Ministry of Economic Development
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Intellectual Property Office of New Zealand

PCT/NZ2004/000275

CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 31 October 2003 with an application for Letters Patent number 529249 made by Auckland UniServices Limited.

Dated 8 November 2004.

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529249

Patents Form No. 4

Our Ref: JC218679

Patents Act 1953

PROVISIONAL SPECIFICATION

NOVEL NITROPHENYL MUSTARD AND AZIRIDINE ALCOHOLS AND THEIR CORRESPONDING PHOSPHATES AND THEIR USE AS BIOREDUCTIVE DRUGS

We, **Auckland UniServices Limited,** a New Zealand company, of Level 10, 70 Symonds Street, Auckland, New Zealand do hereby declare this invention to be described in the following statement:

PT043844247

NOVEL NITROPHENYL MUSTARD AND AZIRIDINE ALCOHOLS AND THEIR CORRESPONDING PHOSPHATES AND THEIR USE AS BIOREDUCTIVE DRUGS

The present invention relates to novel nitrophenyl mustard and aziridine alcohols, to their corresponding phosphates, to their use as bioreductive drugs in hypoxic tumours, and to their use in gene-directed enzyme-prodrug therapy (GDEPT) and antibody-directed enzyme-prodrug therapy (ADEPT), in conjunction with nitroreductase enzymes.

Background to the invention

The use of tumour-selective prodrugs (relatively inactive compounds that can be selectively converted to more active compounds *in vivo*) is a valuable concept in cancer therapy (see, for example Denny, *Eur. J. Med. Chem.* (2001) 36, 577).

For example a prodrug may be converted into an anti-tumour agent under the influence of an enzyme that is linkable to a monoclonal antibody that will bind to a tumour associated antigen. The combination of such a prodrug with such an enzyme monoclonal/antibody conjugate represents a very powerful clinical agent. This approach to cancer therapy, often referred to as "antibody directed enzyme/prodrug therapy" (ADEPT), is disclosed in W088/07378.

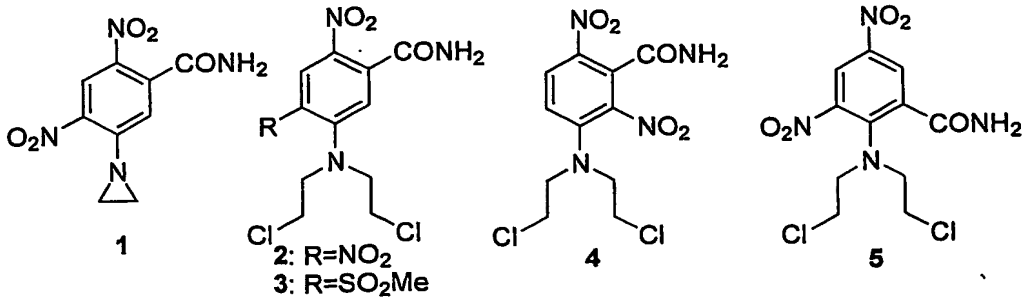
A further therapeutic approach termed "virus-directed enzyme prodrug therapy" (VDEPT) has been proposed as a method for treating tumour cells in patients using prodrugs. Tumour cells are targeted with a viral vector carrying a gene encoding an enzyme capable of activating a prodrug. The gene may be transcriptionally regulated by tissue specific promoter or enhancer sequences. The viral vector enters tumour cells and expresses the enzyme, in order that a prodrug is converted to an active drug within the tumour cells (Huber et al., *Proc. Natl. Acad. Sci.* USA (1991) 88, 8039). Alternatively, non-viral methods for the delivery of genes have been used. Such methods include calcium phosphate co-precipitation, microinjection, liposomes, direct DNA uptake, and receptor-mediated DNA transfer. These are reviewed in

Morgan & French, *Annu. Rev. Biochem.*, 1993, 62; 191. The term "GDEPT" (gene-directed enzyme prodrug therapy) is used to include both viral and non-viral delivery systems.

4-Nitroaromatic compounds are reduced by both mammalian and bacterial flavoprotein enzymes, which effect stepwise addition of up to six electrons. The major enzymatic metabolite is usually the 4-electron species (hydroxylamine).

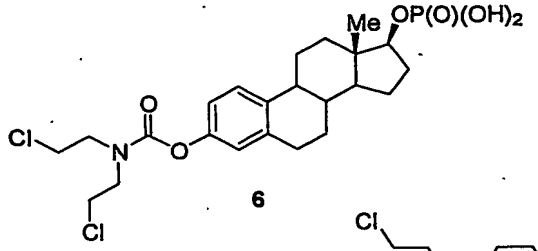
A number of nitrophenyl mustards and nitrophenylaziridines have been reported as prodrugs for use in gene-directed enzyme-prodrug therapy (GDEPT) in conjunction with nitroreductase enzymes. In particular, CB 1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide; (1) [shown below] is reported to be a substrate for the aerobic nitroreductase NTR (nfsB gene product) isolated from *E. coli* B (Boland et al., Biochem. Pharmacol. 1991, 41, 867-875; Anlezark et al., Biochem. Pharmacol, 1992, 44, 2289-2295; Parkinson et al., J. Med. Chem. 2000, 43, 3624). This compound has been used as a prodrug in both ADEPT (Knox et al., Biochem. Pharmacol., 1995, 49, 1641-1647) and GDEPT (Bridgewater et al.,. Eur. J. Cancer, 1995, 31A, 2362-2370; Bailey et al., Gene Ther., 1996, 3, 1143-1150; Bailey and Hart, Gene Ther., 1997, 4, 80-81; Green et al., Cancer Gene Ther., 1997, 4, 229-238) applications, including a clinical trial (Chung-Faye et al., Clin. Cancer Res., 2001, 7, 2662-2668).

Similarly, the dinitrophenyl mustard SN 23862 (**2**) is also a substrate for NTR, and shows selective toxicity towards cell lines that express the enzyme. It is activated by reduction of the 4-nitro group (Palmer et al., J. Med. Chem., 1995, 38, 1229; Kestell et al., Cancer Chemother. Pharmacol., 2000, 46, 365-374). The 4-SO_2Me derivative (**3**) was also a substrate (Atwell et al., Anti-Cancer Drug Des., 1996, 11, 553), as were the regioisomers (**4**) and (**5**) (Friedlos et al., J. Med. Chem., 1997, 40, 1270).



However, compounds of this type were not very effective as bioreductive prodrugs when these compounds were activated in hypoxic tumour tissue by endogenous reductase enzymes, showing ratios of 2-5 fold under hypoxic conditions in the wild-type AA8 cell line, using a clonogenic assay (Palmer et al., J. Med. Chem. 1996, 39, 2518-2528).

Some phosphate analogues of mustards have been described, for the purpose of solubilising the compounds. The best known is estramustine phosphate (Estracyt; **6**), which has been shown to bind to tubulin binding domains on various microtubule-associated proteins (Moraga et al., Biochim. Biophys. Acta, 1992, 1121, 97-103), and which has been shown to be active in advanced breast cancer (Keren-Rosenberg et al., Semin. Oncol., 1997, 24 (Suppl. 3), 26-29). Another study has also shown estramustine phosphate to be a radiation sensitizer (Kim et al., Int. J. Radiat. Oncol. Biol. Phys., 1994, 29, 555-557). The phenol mustard phosphate analogues **7** is a carboxypeptidase substrate (Matsui et al., Japanese Patent 07082280 A2, 1995), and the solubilised mustard **8** has been described as a phosphatase inhibitor (Workman, Chem.-Biol. Interact., 1978, 20, 103-112).



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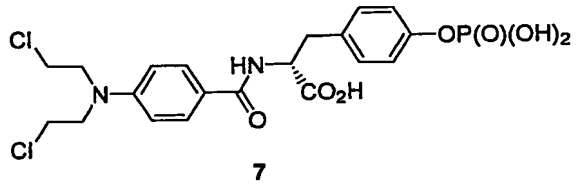

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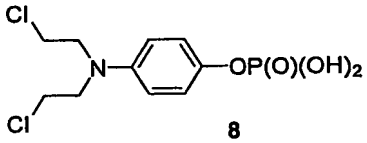

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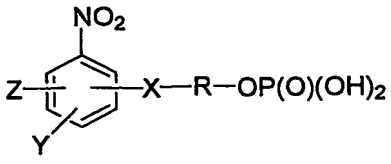
It is an object of the present invention to provide a specific class of nitrophenyl mustards and aziridines, bearing short-chain alcohols, and their corresponding phosphates, as effective bioreductive prodrugs or to at least provide the public with a useful alternative.

Summary and detailed description

In a first aspect, the present invention provides novel phosphate compounds of Formula 1



(I)

wherein:

X may represent at any available ring position –CONH-, -SONH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

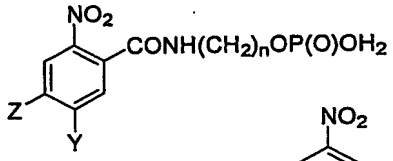
R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

Y may represent at any available ring position -N-aziridinyl or -$N(CH_2CH_2W)_2$, where each W is independently selected from halogen or -OSO_2Me;

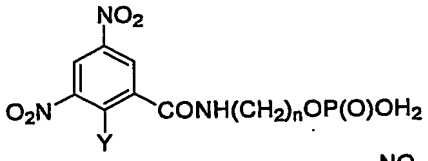
Z may represent at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me;

and pharmaceutically acceptable salts and derivatives thereof.

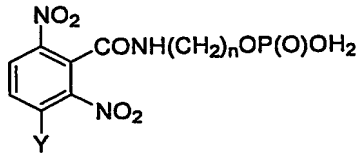
In a preferred embodiment, the phosphate compound of Formula (I) is selected from a compound represented by formulae (Ia), (Ib) or (Ic)



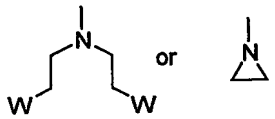
(Ia)



(Ib)



(Ic)

wherein Y may represent 

and wherein

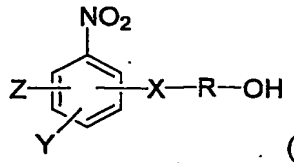
n may represent 1 to 6

Z may represent -NO_2, -halogen, -CN, -CF_3 or -SO_2Me; and

where each W is independently selected from halogen or -OSO_2Me

and pharmaceutically acceptable salts and derivatives thereof.

In a second aspect, the present invention provides alcohol compounds of Formula II



(II)

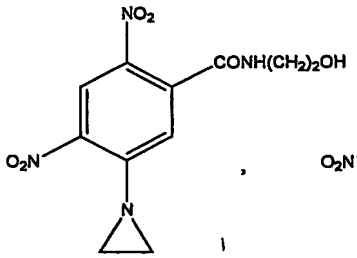
wherein:

X may represent at any available ring position –CONH-, -SONH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

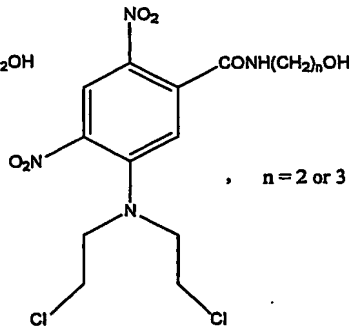
Y may represent at any available ring position -N-aziridinyl or -$N(CH_2CH_2W)_2$, where each W is independently selected from halogen or -OSO_2Me;

Z may represent at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me;

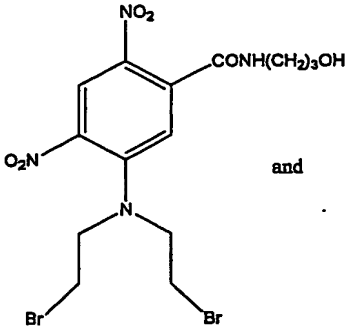
R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, with the proviso that



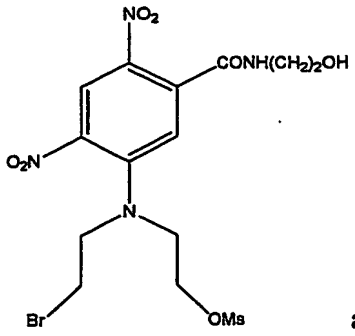
,



, n = 2 or 3

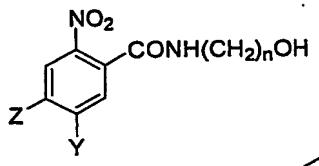


and

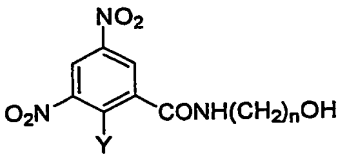


are excluded.

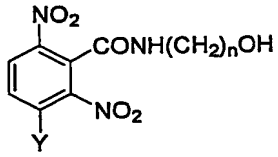
In a preferred embodiment, the alcohol compound of Formula (II) is selected from a compound represented by formulae (IIa), (IIb) or (IIc)



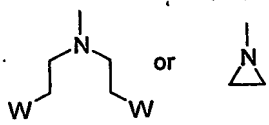
(IIa)



(IIb)



(IIc)

wherein Y may represent 

or

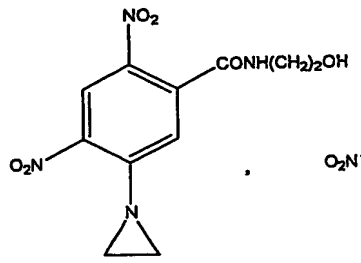
and wherein

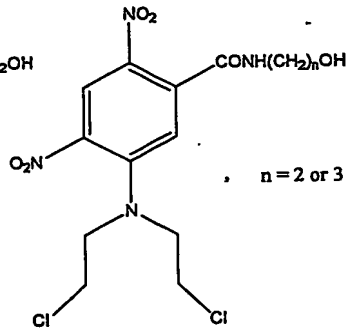
n may represent 1 to 6

Z may represent $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

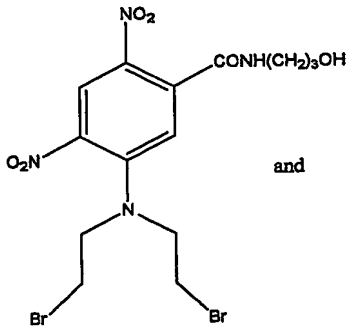
where each W is independently selected from halogen or $-OSO_2Me$

and pharmaceutically acceptable salts and derivatives thereof with the proviso that





n = 2 or 3



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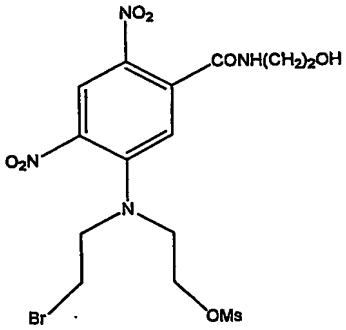
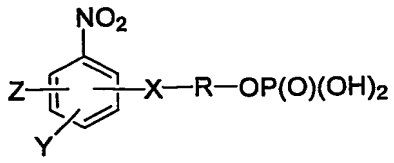


are excluded.

In a third aspect of the invention there is provided a method of preparing the phosphates represented by the general formula (I);



(I)

wherein:

X may represent at any available ring position –CONH-, -SONH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

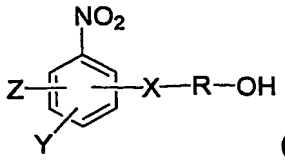
R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

Y may represent at any available ring position -N-aziridinyl or -$N(CH_2CH_2W)_2$, where each W is independently selected from halogen or -OSO_2Me;

Z may represent at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me;

and pharmaceutically acceptable salts and derivatives thereof;
the method including the step of

(i) phosphorylating a compound of formula (II)



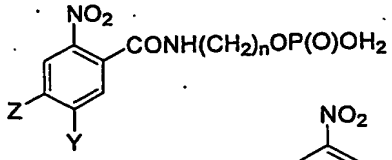
(II)

wherein:
X may represent at any available ring position –CONH-, -SONH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

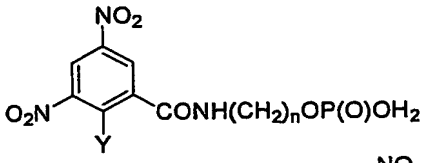
Y may represent at any available ring position -N-aziridinyl or -$N(CH_2CH_2W)_2$, where each W is independently selected from halogen or -OSO_2Me;

Z may represent at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me; and
R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom.

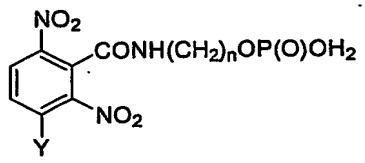
In a preferred embodiment there is provided a method of preparing a compound of formulae (Ia), (Ib) or (Ic)



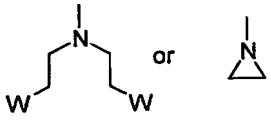
(Ia)



(Ib)



(Ic)

wherein Y may represent  or

and wherein

n may represent 1 to 6

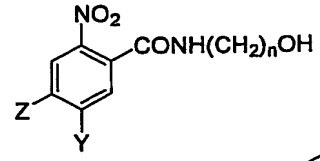
Z may represent $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

where each W is independently selected from halogen or $-OSO_2Me$

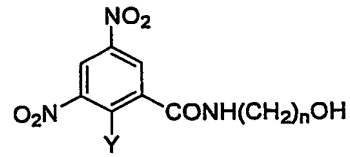
and pharmaceutically acceptable salts and derivatives thereof

the method including the step of

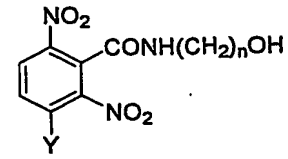
phosphorylating a compound represented by formulae (IIa), (IIb) or (IIc)



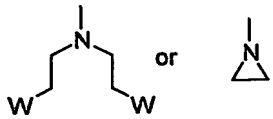
(IIa)



(IIb)



(IIc)

wherein Y may represent  or

and wherein

n may represent 1 to 6

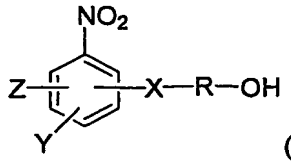
Z may represent $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$; and

where each W is independently selected from halogen or $-OSO_2Me$

and pharmaceutically acceptable salts and derivatives.

In a fourth aspect there is provided a compound of formula (I), formula (Ia), (Ib) or (Ic) obtained by any one of the preparative methods defined above.

In a fifth aspect, the present invention provides a method for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as hypoxia-selective cytotoxins, including the step of administering a compound of Formula I as defined above or a compound of Formula II



(II)

wherein:

X may represent at any available ring position –CONH-, -SONH-, -O-, $-CH_2-$, -NHCO- or $-NHSO_2-$;

Y may represent at any available ring position -N-aziridinyl or $-N(CH_2CH_2W)_2$, where each W is independently selected from halogen or $-OSO_2Me$;

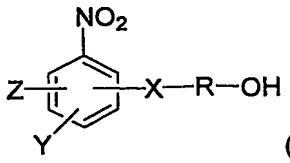
Z may represent at any available ring position $-NO_2$, -halogen, -CN, $-CF_3$ or $-SO_2Me$;

R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof;

or a mixture thereof in a "therapeutically effective amount" to tumour cells in a subject.

Preferably, the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

In a sixth aspect, the present invention provides a method for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as an anticancer agent including the step of administering a compound of Formula I as defined above or a compound of Formula II



(II)

wherein:

X may represent at any available ring position –CONH-, -SONH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

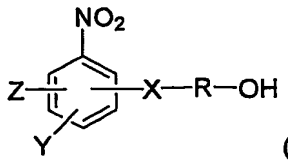
Y may represent at any available ring position -N-aziridinyl or -$N(CH_2CH_2W)_2$, where each W is independently selected from halogen or -OSO_2Me;

Z may represent at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me;

R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof;
or a mixture thereof in a "therapeutically effective amount" to target tumour cells in a subject.

Preferably the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

In a seventh aspect of the present invention, there is provided a method of cell ablation therapy utilising at least one nitroreductase enzyme, wherein the method includes the step of administering a compound of Formula I as defined a above or a compound of Formula II



(II)

wherein:

X may represent at any available ring position –CONH-, -SONH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

Y may represent at any available ring position -N-aziridinyl or -$N(CH_2CH_2W)_2$, where each W is independently selected from halogen or -OSO_2Me;

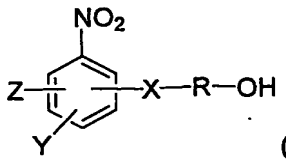
Z may represent at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me;

R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, or a mixture thereof in a "therapeutically effective amount" to ablate tumour cells in tissue in a subject, wherein said tissue expresses the at least one nitroreductase enzyme.

Preferably the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

Preferably, the cell ablation therapy provides a substantially minimal bystander effect.

In an eighth aspect of the present invention there is provided a pharmaceutical composition including a therapeutically effective amount of a compound of Formula I or a compound of Formula II



(II)

wherein:

X may represent at any available ring position –CONH-, -SONH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

Y may represent at any available ring position -N-aziridinyl or -$N(CH_2CH_2W)_2$, where each W is independently selected from halogen or -OSO_2Me;

Z may represent at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me;

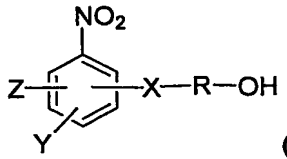
R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof,
or a mixture thereof, and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

The pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser should preferably be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, such as cutaneous, subcutaneous, or intravenous. It is to be appreciated that these factors could be readily determined by someone skilled in the art without undue experimentation.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvent. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such as gelatin.

For intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has a suitable pH, isotonicity and stability. Those of relevent skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride injection, Ringer's injection, Lactated Ringer's injection. Preservatives, stabilisers, buffers antioxidants and/or other additives may be included as required.

In a ninth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined above or a compound of Formula II



(II)

wherein:

X may represent at any available ring position –CONH-, -SONH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

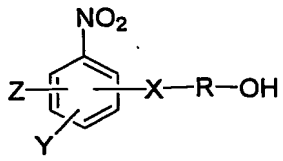
Y may represent at any available ring position -N-aziridinyl or -$N(CH_2CH_2W)_2$, where each W is independently selected from halogen or -OSO_2Me;

Z may represent at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me;

R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, or mixtures thereof,

for use in GDEPT to target cancer cells in a subject in need thereof.

In a tenth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined above or a compound of Formula II



(II)

wherein:

X may represent at any available ring position –CONH-, -SONH-, -O-, -CH_2-, -NHCO- or -$NHSO_2$-;

Y may represent at any available ring position -N-aziridinyl or -$N(CH_2CH_2W)_2$, where each W is independently selected from halogen or -OSO_2Me;

Z may represent at any available ring position -NO_2, -halogen, -CN, -CF_3 or -SO_2Me;

R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof,
or mixtures thereof for use in cell ablation therapy to target cancer cells in a subject in need thereof.

While the compounds of the present invention will typically be used to target tumour cells or tumour tissues in human subjects, they may be used to target tumour cells or tissues in other warm blooded animal subjects such as other primates, farm animals such as cattle, and sports animals and pets such as horses, dogs, and cats.

As used throughout the specification the term "therapeutically effective amount", is to be understood as an amount of a compound of Formula I or Formula II as defined above or a compound of any one of compounds Ia-IIc, or IIa-IIc as defined above or a mixture thereof that is sufficient to show benefit to a subject with cancer cells. The actual amount, rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment is within the responsibility of general practitioners and other medical doctors.

It is to be understood that the compounds of the invention as defined above may be administered alone or in combination with other treatments, especially radiotherapy, either simultaneously or sequentially dependent upon the condition to be treated.

As used throughout the specification the pharmaceutically acceptable derivatives and salts thereof include acid derived salts formed from are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isethionic acids and the like and base derived salts formed from sodium and potassium carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine and the like.

The technique of cell ablation therapy, would be known to someone skilled in the art. This therapy can be used to selectively ablate specified target cells or tissue through specific enzymatic expression of a nitroreductase for example, that is specifically expressed by the tissue and which can then be employed to active a prodrug into an active metabolite to ablate the specified target cells or tissue. (Gusterson *et al.* *Endocrine Related Cancer*, 1997, 4, 67-74.)

The expression "substantially minimal bystander effect" is to be understood as meaning that the killing of adjoining non-targeted tumour cells is minimal as a result of diffusion between the targeted tumour cells and non-targeted tumour cells of an activated metabolite that arises from the enzymatic activation of a compound of Formula I or Formula II as defined above or a compound of any one of compounds Ia-Ic, or IIa-IIc as defined above or a mixture thereof.

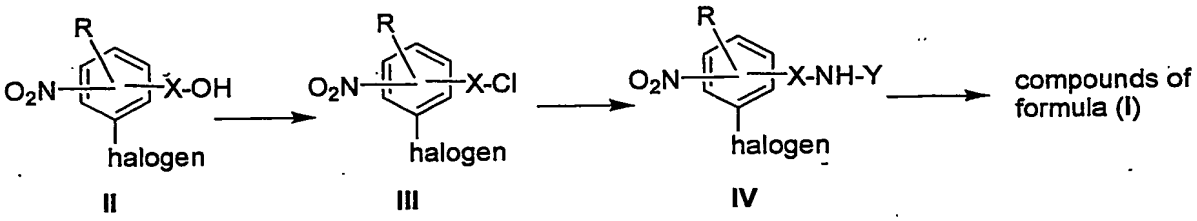
Pharmaceutically acceptable salts of formula (I) include the basic or acidic compounds of formula (I) that form pharmaceutically acceptable salts with both organic and inorganic acids and/or organic and inorganic bases. Examples of suitable acids for salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isethionic, and the like. Examples of suitable

bases for salt formation are sodium and potassium carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine, and the like.

Further aspects of the present invention will become apparent from the following description given by way of example only and with reference to the accompanying synthetic schemes.

The compounds of Formula I can be prepared by the processes described in Scheme 1, where

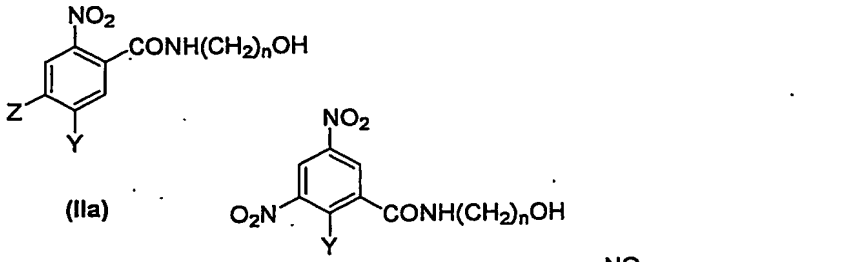
Scheme 1



II III IV

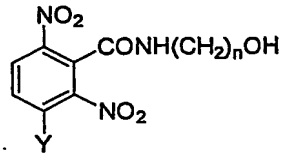
X, Y and R are as defined above for formula (I).

The following Tables 1a and 2a set out physical data for compounds within the general Formula (I) and (II), representative of it, and capable of being prepared by the processes of the invention.



(IIa)

(IIb)



(IIc)

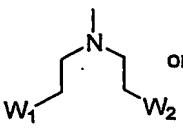
wherein Y may represent  or 

Table 1a. Representative examples of parent alcohols

No	Z	Y	Y (W_1,W_2)	n	mp	formula or ref	analyses
IIa-1	NO_2	aziridine	-	2	192-193	Ref. 1	C, H, N
IIa-2	NO_2	-	Cl,Cl	2		Ref. 2	
IIa-3	NO_2	-	Cl,Cl	3	90-91	Ref 4	C, H, N, Cl
IIa-7	NO_2	-	Br,Br	2	151-152	$C_{13}H_{16}Br_2N_4O_6$	C, H, N, Br
IIa-7s	SO_2Me	-	Br,Br	2	126-127	$C_{14}H_{19}Br_2N_3O_6S$	C, H, N
IIa -8	NO_2	-	Br,Br	3	85-86	Ref 4	C, H, N, Br
IIa-9	NO_2	-	Br,Br	4	123-124	$C_{15}H_{20}Br_2N_4O_6$	C, H, N, Br
IIa-10	NO_2	-	Br,Br	5	gum	$C_{16}H_{22}Br_2N_4O_6$	HRMS
IIa-11	NO_2	-	Br,Br	6	gum	$C_{17}H_{24}Br_2N_4O_6$	HRMS
IIa-12	NO_2	-	Br,OMs	2		Ref. 2	
IIa-13	NO_2	-	Br,OMs	3	gum	$C_{16}H_{21}BrN_4O_9S$	HRMS
IIa 14	NO_2	-	I,I	2	142-143	$C_{13}H_{16}I_2N_4O_6$	C, H, N, I
IIb -1	-	aziridine	-	6	189-192	$C_{15}H_{20}N_4O_6$	C, H, N
IIb -2	-	-	Cl,Cl	2	109-111	$C_{13}H_{16}Cl_2N_4O_6$	C, H, N
IIb-3	-	-	Cl,Cl	3	89-91	$C_{14}H_{18}Cl_2N_4O_6$	C, H, N, Cl
IIb-4	-	-	Cl,Cl	4	gum	$C_{15}H_{20}Cl_2N_4O_6$	HRMS
IIb-5	-	-	Cl,Cl	5	gum	$C_{16}H_{22}Cl_2N_4O_6$	HRMS
IIb-6	-	-	Cl,Cl	6	gum	$C_{17}H_{24}Cl_2N_4O_6$	HRMS
IIb-7	-	-	Br,Br	2	105-108	$C_{13}H_{16}Br_2N_4O_6$	C, H, N, Br
IIb-7a	-	-	Br,Br[A]	2	127-130	$C_{15}H_{20}Br_2N_4O_6$	C, H, N
IIb -8	-	-	Br,Br	3	89-94	$C_{14}H_{18}Br_2N_4O_6$	C, H, N, Br
IIb -9	-	-	Br,Br	4	gum	$C_{15}H_{20}Br_2N_4O_6$	HRMS
IIb-10	-	-	Br,Br	5	gum	$C_{16}H_{22}Br_2N_4O_6$	HRMS
IIb-11	-	-	Br,Br	6	gum	$C_{17}H_{24}Br_2N_4O_6$	HRMS
IIb-12	-	-	Br,OMs	2		Ref. 3	
IIb-13	-	-	Br,OMs	3	gum	$C_{15}H_{21}BrN_4O_9S$	HRMS

IIb-14	-	-	I,I	2	129-131	$C_{13}H_{16}I_2N_4O_6$	C,H,N
IIb-15	-	-	I,OMs	2	gum	$C_{14}H_{19}IN_4O_9S$	HRMS
IIc-7	-	-	Br,Br	2	gum	$C_{13}H_{16}Br_2N_4O_6$	HRMS
IIc-8	-	-	Br,Br	3	gum	$C_{16}H_{22}Br_2N_4O_6$	HRMS
IIc-9	-	-	Br,Br	4	gum	$C_{17}H_{24}Br_2N_4O_6$	HRMS
IIc-12	-	-	Br,OMs	2	94-97	$C_{14}H_{19}BrN_4O_9S$	C, H, N
IIc-13	-	-	Br,OMs	3	115-117	Ref. 3	C, H, N
IIc-14	-	-	Br,OMs	4	114-117	$C_{16}H_{23}BrN_4O_9S$	C, H, N

[A]α-methyl mustard

Notes

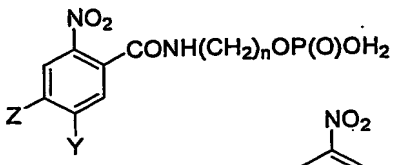
References for known compounds.

1. Khan AH, Ross WCJ. Tumor-growth inhibitory nitrophenylaziridines and related compounds. Structure-activity relations. II. Chem.-Biol. Int., 1971, 4, 11-22.

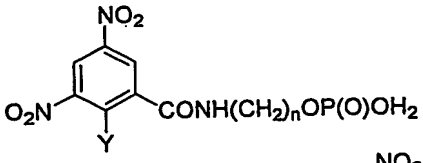
2 NZ Patent No.240785

3. Co-pending NZ Application No. 521851

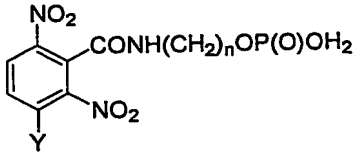
4. Wilson WR, Pullen SM, Hogg A, Helsby NA, Hicks KO, Denny WA. Quantitation of bystander effects in nitroreductase suicide gene therapy using three-dimensional cell cultures. Cancer Res., 2002, 62, 1425-1432.



(Ia)



(Ib)



(Ic)

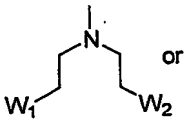
wherein Y may represent  or 

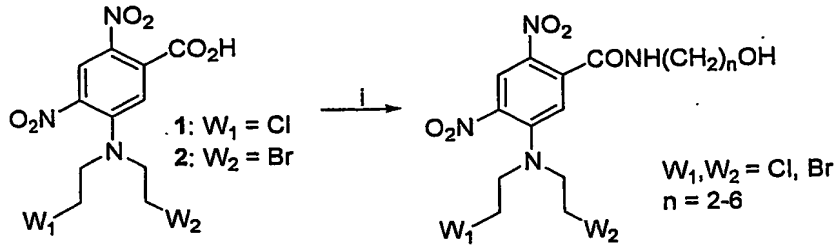
Table 1b. Examples of phosphates of formulae Ia-Ic

No	Z	Y (W_1, W_2)	n	mp	formula	analyses
Ia-3P	NO_2	Cl,Cl	3	195-200	$C_{14}H_{19}Cl_2N_4O_9P$	HRMS
Ia-8P	NO_2	Br,Br	3	170-174	$C_{14}H_{17}Cl_2N_4O_9P$	HRMS
Ib-2P	-	Cl,Cl	2	Foam		HRMS
Ib-7P	-	Br,Br	2	Foam	$C_{13}H_{17}Br_2N_4O_9P$	HRMS
Ib-7aP	-	Br,Br[A]	2	157-161	$C_{15}H_{21}Br_2N_4O_9P$	C, H, N
Ib-12P	-	Br,Oms	2	Foam	$C_{14}H_{21}BrN_4O_{12}PS$	HRMS
Ib-14P	-	I,I	2	Foam		HRMS
Ib-15P	-	I,Oms	2	147-150	$C_{14}H_{20}IN_4O_{12}PS$	C, H, N
Ic-8P	-	Br,Br	3	Foam		HRMS
Ic-13P	-	Br,Oms	3	foam		HRMS

[A]alpha-Me

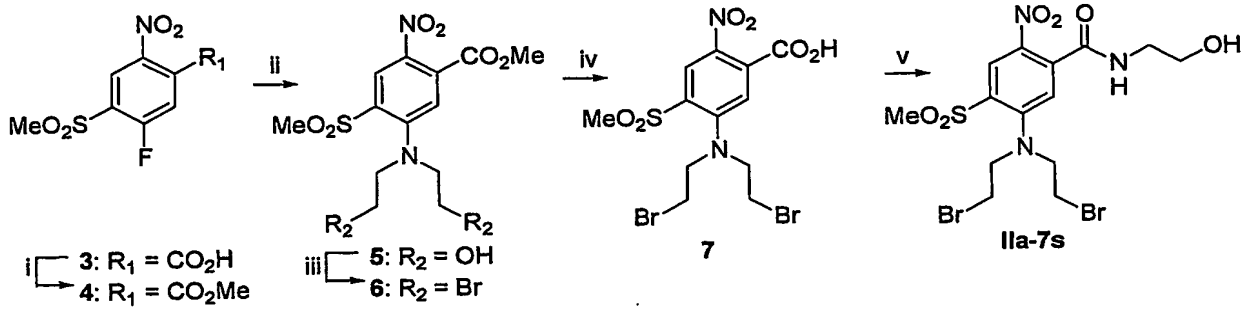
The compounds of Table 1a can be prepared by the general methods set out in Schemes 2a-2h, and exemplified in Examples 1-20 below.

Scheme 2a



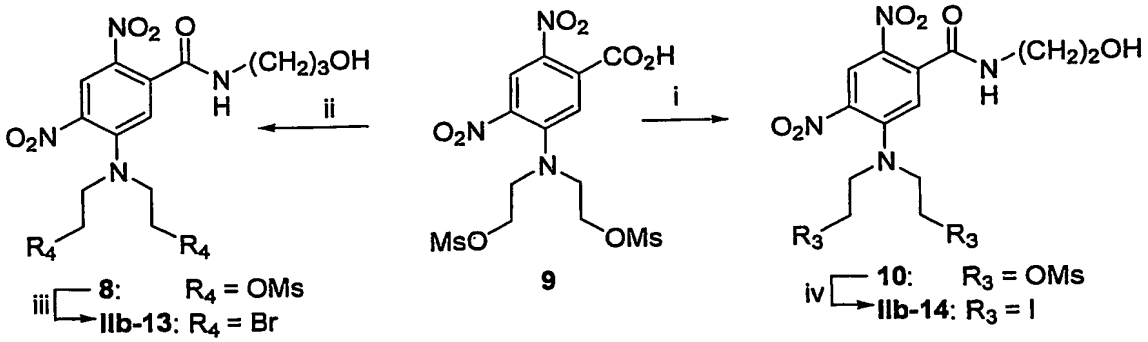
(i) $SOCl_2$ or oxalyl bromide, then $HO(CH_2)_nNH_2$.

Scheme 2b



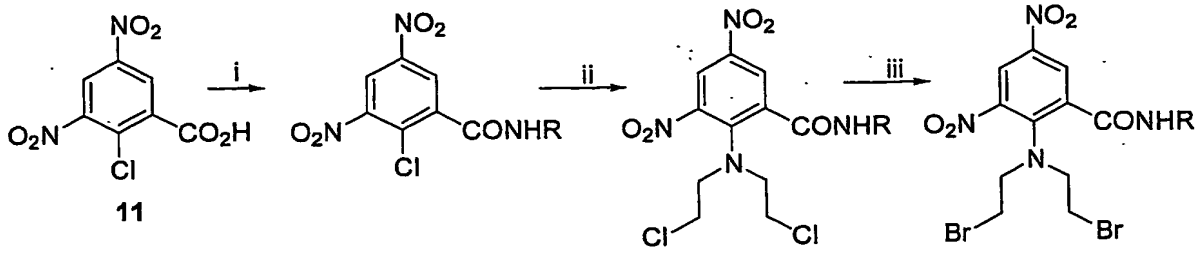
(i) $SOCl_2$/MeOH;
(ii) diethanolamine/DMA;
(iii) MsCl/py, then NaBr/DMF;
(iv) KOH;
(v) $SOBr_2$/benzene, then 2-aminoethanol

Scheme 2c



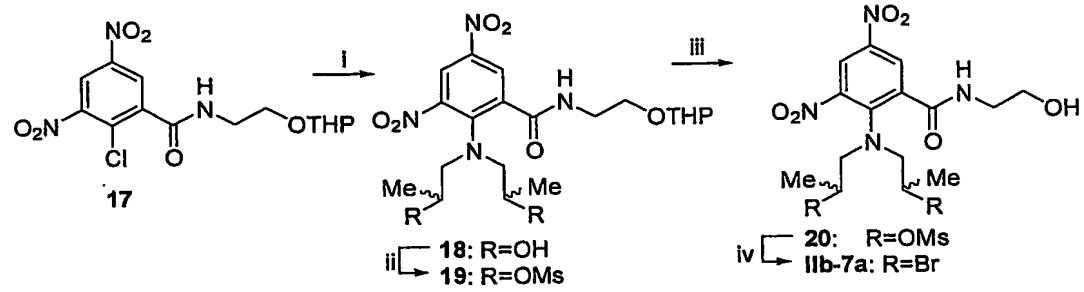
(i) $SOCl_2$/MeOH, then 2-aminoethanol
(ii) $SOCl_2$/MeOH, then 3-amino-1-propanol
(iii) LiBr (1.4 equiv.)/DMF;
(iv) NaI (excess)/MeCN.

Scheme 2d



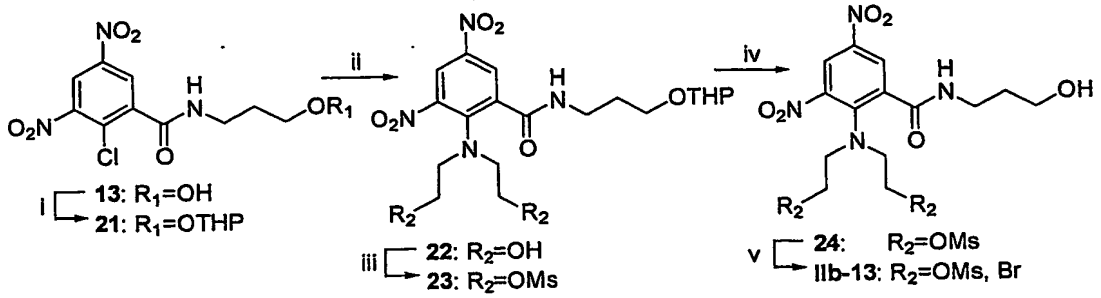
(i) $SOCl_2$/cat. DMF, then RNH_2;
(ii) $HN(CH_2CH_2Cl)_2$;
(iii) LiBr/3-Mebutanone

Scheme 2e



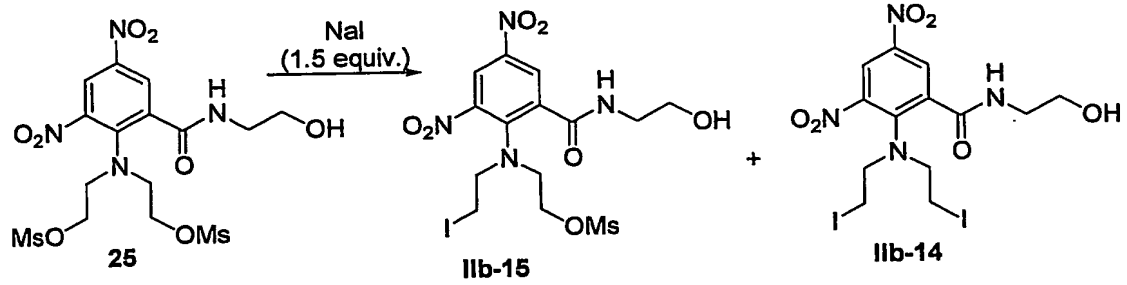
(i) diisopropanolamine;
(ii) MsCl/Et_3N;
(iii) 1N HCl/THF;
(iv) LiBr (excess).

Scheme 2f

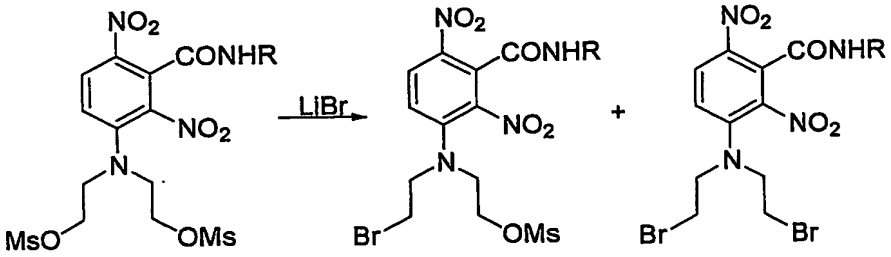


(i) 3,4-dihydro-2*H*-pyran, *p*-TosOH;
(ii) diethanolamine;
(iii) $MsCl/Et_3N$;
(iv) 1N HCl/THF;
(v) LiBr (1.2eq).

Scheme 2g



Scheme 2h



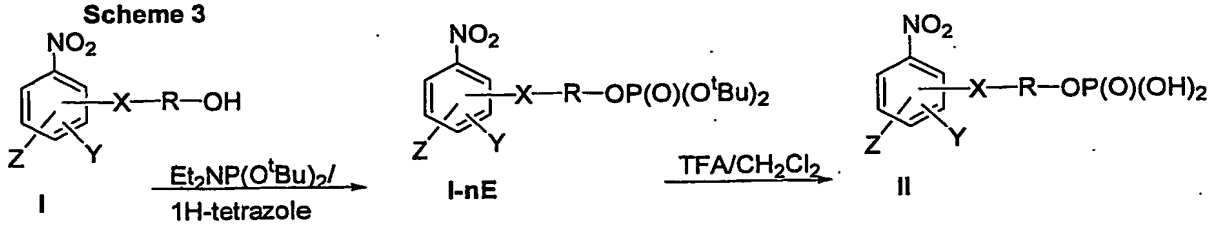
26: R=$(CH_2)_2OH$
27: R=$(CH_2)_3OH$
28: R=$(CH_2)_4OH$

IIc-7: R=$(CH_2)_2OH$
IIc-8: R=$(CH_2)_3OH$
IIc-9: R=$(CH_2)_4OH$

IIc-12: R=$(CH_2)_2OH$
IIc-13: R=$(CH_2)_3OH$
IIc-14: R=$(CH_2)_4OH$

The compounds of Table 1b can be prepared by the general methods set out in Scheme 3, and exemplified in Examples A-G below.

Scheme 3



In Scheme 3, X, Y, Z, and R are as specified for formula (I) and (II) above.

Examples

The invention and the best mode for practising the same are illustrated by the following Examples 1-20 (alcohols) and Examples 21-30 (phosphates).

Example 1 (Scheme 2a). N-(3-Hydroxypropyl)-5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (IIa-3). A suspension of 5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzoic acid [Palmer et al., J. Med. Chem., 1994, 37, 2175] (**1**) (2.50 g, 7.1 mmol) in $SOCl_2$ (20 mL) containing DMF (2 drops) was heated under reflux for 1 h, then concentrated to dryness under reduced pressure and re-evaporated with benzene. The resulting crude benzoyl chloride was dissolved in Me_2CO (50 mL) and the cooled (-5 °C) solution was treated with a cold solution of 3-amino-1-propanol (1.09 g, 14.5 mmol) in water (25 mL). The reaction mixture was shaken at room temperature for 5 min, then diluted with water (25 mL), concentrated to half volume, and extracted with CH_2Cl_2 (2x). The organic extract was washed with 0.1 N HCl and water then worked up to give a solid which was chromatographed on silica gel, eluting with EtOAc to give **IIa-3** (2.37 g, 82%): mp (EtOAc/i-Pr_2O) 90-91°C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.63 (t, J = 5.6 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.42 (s, 1 H, H-6), 4.46 (t, J = 5.1 Hz, 1 H, OH), 3.82 (t, J = 5.9 Hz, 4 H, $N(CH_2CH_2Cl)_2$), 3.68 (t, J = 5.9 Hz, 4 H, $N(CH_2CH_2Cl)_2$), **3.49 (q, J = 6.0 Hz, 2** H, CH_2OH), 3.29 (q, partially obscured, J = 5.9 Hz, 2 H, $CONHCH_2$), 1.68 (pent, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{14}H_{18}Cl_2N_4O_6$) C, H, N, Cl.

Example 2 (Scheme 2a). N-(3-Hydroxypropyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa-8). A suspension of powdered 5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzoic acid (**2**) (1.10 g, 2.49 mmol) in benzene (170 mL) was treated at 20 °C with oxalyl bromide (1.10 mL, 11.7 mmol) and DMF (2 drops). The mixture was stirred at 20 °C for 2 h, then concentrated under reduced pressure, and re-evaporated to dryness in the presence of benzene under high vacuum. The resulting acid bromide was dissolved in Me_2CO (20 mL) and the solution was treated at –5 °C with a cold solution of 3-amino-1-propanol (0.39 g, 5.19 mmol) in water (10 mL). The mixture was shaken at room temperature for 5 min, then diluted with water and extracted with EtOAc (2x). The organic extract was worked up and the resulting residue was chromatographed on silica gel, eluting with EtOAc, to give **IIa-8** (1.06 g, 85%): mp (EtOAc/i-Pr_2O) 85-86 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.64 (t, J = 5.6 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.41 (s, 1 H, H-6), 3.77-3.64 (m, 8 H, $N(CH_2CH_2Cl)_2$), 4.46 (br s, 1 H, OH), 3.49 (t, J = 6.3 Hz, 2 H, CH_2OH), 3.33-3.25 (m, partially obscured, 2 H, $CONHCH_2$), 1.68 (pent, J = 6.72 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{14}H_{18}Br_2N_4O_6$) C, H, N, Br.

Example 3 (Scheme 2a). N-(2-Hydroxyethyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa-7). Similar reaction of **2** with 2-aminoethanol gave **IIa-7** (0.78 g, 46%): mp (MeOH/EtOAc/pet. ether) 151-152 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (t, J = 5.7 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.43 (s, 1 H, H-6), 4.76 (t, J = 5.6 Hz, 1 H, OH), 3.77-3.64 (m, 8 H, $N(CH_2CH_2Br)_2$), 3.53 (q, J = 6.0 Hz, 2 H, CH_2OH), 3.31 (q, partially obscured, J = 6.1 Hz, 2 H, $CONHCH_2$). Anal. ($C_{13}H_{16}Br_2N_4O_6$) C, H, N, Br.

Example 4 (Scheme 2a). N-(4-Hydroxybutyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa -9). Similar reaction of **2** with 4-amino-1-butanol in cold Me_2CO, followed by chromatography on silica gel and elution with EtOAc gave **IIa-9** (69%) as a yellow solid: mp (EtOAc/iPr_2O) 123-124 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.62 (t, J = 5.6 Hz, 1 H), 8.53 (s, 1 H), 7.39 (s, 1 H), 4.39 (t, J = 5.1 Hz, 1 H), 3.78-3.64 (m, 8 H), 3.47-3.40 (m, 2 H), 3.27-3.20 (m, 2 H), 1,61-1.44 (m, 4 H). Anal. ($C_{15}H_{20}Br_2N_4O_6$) C, H, N, Br.

Example 5 (Scheme 2a). N-(5-Hydroxypentyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa -10). Similar reaction of **2** with 5-amino-1-pentanol in cold Me_2CO,

followed by chromatography on silica gel and elution with EtOAc gave **IIa -10** (66%) as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.62 (t, J = 5.6 Hz, 1 H), 8.53 (s, 1 H), 7.38 (s, 1 H), 4.34 (t, J = 5.1 Hz, 1 H), 3.79-3.64 (m, 8 H), 3.44-3.37 (m, 2 H), 3.26-3.18 (m, 2 H), 1.59-1.29 (m, 4 H). HRMS (FAB) Calcd. for $C_{16}H_{23}{}^{79}Br_2N_4O_6$ [M+H$^+$] m/z 524.9984, found 524.9964.

Example 6 (Scheme 2a). N-(6-Hydroxyhexyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa -11). Similar reaction of **32** with 6-amino-1-hexanol in cold Me_2CO, followed by chromatography on silica gel and elution with EtOAc gave **IIa -11** (72%) as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.61 (t, J = 5.6 Hz, 1 H), 8.53 (s, 1 H), 7.38 (s, 1 H), 4.31 (t, J = 5.2 Hz, 1 H), 3.79-3.64 (m, 8 H), 3.43-3.36 (m, 2 H), 3.27-3.19 (m, 2 H), 1.58-1.26 (m, 4 H). HRMS (FAB) Calcd. for $C_{17}H_{25}{}^{79}Br_2N_4O_6$ [M+H$^+$] m/z 539.0141, found 539.0137.

Example 7. (Scheme 2b). 5-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-(methylsulfonyl)-2-nitrobenzamide (IIa -7s). 5-Fluoro-4-(methylsulfonyl)-2-nitrobenzoic acid [Atwell et al., ACDD, 1996, 11, 553] (**3**) was heated in excess $SOCl_2$/catalytic DMF to provide the acid chloride, which was reacted with dry MeOH to give methyl 5-fluoro-4-(methylsulfonyl)-2-nitrobenzoate (**4**): mp (EtOAc/hexane) 134-135 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.49 (d, J = 5.9 Hz, 1 H), 8.14 (d, J = 9.3 Hz, 1 H), 3.92 (s, 3 H), 3.46 (s, 3 H). Anal. ($C_9H_8FNO_6S$) C, H, N.

A mixture of **4** (1.48 g, 5.34 mmol) and diethanolamine (1.40 g, 13.3 mmol) in DMA (6 mL) was stirred at 30 °C for 1 h, and then diluted with EtOAc (60 mL). The solution was washed with brine (2x) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH, followed by recrystallization from EtOAc/iPr_2O, to give methyl 5-[bis(2-hydroxyethyl)amino]-4-(methylsulfonyl)-2-nitrobenzoate (**5**) (1.41 g, 73%): mp 99-100 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.56 (s, 1 H), 7.73 (s, 1 H), 4.62 (t, J = 4.9 Hz, 2 H), 3.89 (s, 3 H), 3.59-3.49 (m, 8 H), 3.45 (s, 3 H). Anal. ($C_{13}H_{18}N_2O_8S$) C, H, N.

A solution of **5** (1.48 g, 4.08 mmol) in dry pyridine (15 mL) was treated dropwise at 0 °C with MsCl (0.80 mL, 10.3 mmol). The reaction was stirred at 0 °C for 2 h, then poured into 10% aqueous NaBr. The resulting crude dimesylate was collected, washed well with water, dried, dissolved in DMF (15 mL) and stirred with NaBr (21.6 g, 25 mmol) at 70 °C for 1.5 h. The cooled mixture was poured into water and the resulting solid was purified by chromatography, on silica gel, eluting with CH_2Cl_2, then recrystallisation from CH_2Cl_2/iPr_2O to give methyl 5-[bis(2-bromoethyl)amino]-4-(methylsulfonyl)-2-nitrobenzoate (**6**) (1.47 g, 74%): mp 161-162 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.58 (s, 1 H), 7.94 (s, 1 H), 3.90 (s, 3 H), 3.82 (t, J = 7.0 Hz, 4 H), 3.63 (t, J = 6.9 Hz, 4 H), 3.48 (s, 3 H). Anal. ($C_{13}H_{16}Br_2N_2O_6S$) C, H, N.

A solution of **6** (1.00 g, 2.05 mmol) in a dioxane/MeOH (1:1, 20 mL) was treated at 10 °C with 4N aqueous KOH (5 mL), and stirred at 10 °C for 45 min. The mixture was acidified to pH 2 with 1 N aqueous HBr, concentrated to a small volume under reduced pressure, and then diluted with saturated aqueous NaBr (20 mL). The resulting semi-solid was isolated and crystallized twice from MeOH/H_2O to give 5-[bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-(methylsulfonyl)-2-nitrobenzoic acid (**7**) (0.70 g, 72%): mp 174-176 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.50 (s, 1 H), 7.88 (s, 1 H), 3.79 (t, J = 7.0 Hz, 4 H), 3.62 (t, J = 7.0 Hz, 4 H), 3.48 (s, 3 H). Anal. ($C_{12}H_{14}Br_2N_2O_6S$) C, H, N.

A finely-divided suspension of **7** (260 mmg, 0.55 mmol) in dry benzene (50 mL) was treated with $SOBr_2$ (2.13 mL, 0.20 mmol) and catalytic DMF. The mixture was stirred for 2 h, then concentrated to dryness under reduced pressure and re-evaporated with benzene under high vacuum. The resulting crude acid bromide was dissolved in Me_2CO (10 mL) and treated at −5 °C with a cold solution of 2-aminoethanol (101 mg, 1.65 mmol) in water (5 mL). The mixture was stirred at 0 °C for 5 min, then acidified to pH 4 with 1 N aqueous HBr, and concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with EtOAc, to give **IIa -7s** (222 mg, 78%): mp (EtOAc/iPr_2O) 126-127 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.75 (t, J = 5.6 Hz, 1 H), 8.51 (s, 1 h), 7.68 (s, 1 H), 4.79 (t, J = 5.4 Hz, 1 H), 3.76 (t, J = 7.1 Hz, 4 H), 3.62 (t, J = 7.0 Hz, 4 H), 3.54 (q, J = 5.9 Hz, 2 H), 3.48 (s, 3 H), 3.31 (after D_2O exchange, t, J = 6.0 Hz, 2 H). HRMS (FAB) calcd. for $C_{14}H_{20}{}^{79}Br_2N_3O_6S$ (MH^+) *m/z* 515.9440; found 515.9425.

Example 8 (Scheme 2c). 2[(2-Bromoethyl)-5-[[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino]ethyl methanesulfonate (IIa-13) and 5-[bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-2, 4-dinitrobenzamide (IIa-14). 5-(Bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,4-dinitrobenzoic acid [A method of preparing this compound is disclosed in co-pending NZ Application No. 521851] (**9**) was heated under reflux in excess $SOCl_2$ (60 mL) and catalytic DMF for 1 h. Evaporation under reduced pressure, followed by azeotroping in benzene, gave the crude acid chloride. This was dissolved in dry Me_2CO and treated at 0 °C with 3-amino-1-propanol at 0 °C for 5 min. The mixture was acidified to pH 2-3 with 0.2 N HCl, concentrated to half volume, and then solid NaBr was added, followed by extraction with EtOAc (2x). Evaporation, and chromatography of the residue on silica gel, eluting with EtOAc/MeOH (9:1), gave give 2-(5-{[(3-hydroxypropyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**8**) (68%) as a yellow gum; ^{1}H NMR [$(CD_3)_2SO$] δ 8.54 (t, J = 5.7 Hz, 1 H), 8.53 (s, 1 h), 7.45 (s, 1 H), 4.43 (t, J = 5.1 Hz, 1 H), 4.33 (t, J = 5.2 Hz, 4 H), 3.69 (t, J = 5.2 Hz, 4 H), 3.5 7(q, J = 5.9 Hz, 2 H), 3.26 (after D_2O exchange, t, J = 7.0 Hz, 1 H), 3.12 (s, 6 H), 1.66 (pent, J = 6.7 Hz, 2 H). HRMS (FAB) calcd. for $C_{16}H_{25}N_4O_{12}S$ (MH^+) *m/z* 529.0910; found 529.0904.

A solution of **8** in DMF was treated with LiBr (1.4 equiv.), and worked up as above, and the product was chromatographed on silica gel. Elution with EtOAc gave a small amount of the dibromo mustard, while elution with EtOAc/MeOH (19:1) gave **IIa -13** (31%) as a yellow gum: ^{1}H NMR [$(CD_3)_2SO$] δ 8.60 (t, J = 5.6 Hz, 1 H), 8.54 (s, 1 H), 7.44 (s, 1 H), 4.45 (t, J = 5.2 Hz, 1 H), 4.33 (t, J = 5.1 Hz, 2 H), 3.74 (t, J = 5.2 Hz, 2 H), 3.72-3.66 (m, 4 H), 3.49 (q, J = 5.9 Hz, 2 H), 3.27 (after D_2O exchange, t, J = 7.0 Hz, 2 H), 3.14 (s, 3 H), 1.68 (pent, J = 6.7 Hz, 2 H). HRMS (FAB) calcd. for $C_{15}H_{22}{}^{79}BrN_4O_9S$ (MH^+) *m/z* 515.0270; found 515.0283.

Similar treatment of **9** with 2-aminoethanol gave 2-(5-{[(2-hydroxyethyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**10**). A stirred mixture of **10** (1.42 g, 2.76 mmol) and NaI (3.3g, 22 mmol) in dry MeCN (45 mL) was heated at reflux for 1h, then concentrated under reduced pressure. The residue was partitioned between

EtOAc and water, and the organic layer was washed with water and evaporated. The residue was chromatographed on silic gel, eluting with CH_2Cl_2/EtOAc (1:4), followed by recrystallisation from MeOH/EtOAc/i-Pr_2O to give **IIa -14** (2.9 g, 81%): mp 142-143 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (t, ^{J} = 5.7 Hz, 1 H), 8.53 (s, 1 H), 7.38 (s, 1 H), 4.76 (t, J = 5.5 Hz, 1 H), 3.68 (t, J = 6.9 Hz, 4 H), 3.57-3.49 (m, 2 H), 3.39 (t, J = 6.9 Hz, 4 H), 3.34-3.26 (m, partially obscured, 2 H). Anal. ($C_{13}H_{16}I_2N_4O_6$) C, H, N.

Example 9. 2-Chloro-2-[bis(2-chloroethyl)amino]-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (IIa -1). A solution of 2-chloro-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (**14**) [for preparation see Example 14 below] (118 mg, 0.34 mmol) and Et_3N (200 mg) in EtOAc (200 mL) was treated with aziridine (100 mg) at room temperature for 3 h. The mixture was diluted with EtOAc and washed three times with water, after dry, concentrated under reduced pressure until about 20 mL, the yellow solid was collected and gave 101 mg product (84%); ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.8 Hz, 1 H), 8.63 (m, 1 H), 8.29 (d, J = 2.8 Hz, 1 H), 4.31 (m, 1 H), 3.39 (m, 2 H), 3.25 (m, 2 H), 2.37 (s, 4 H), 1.56 (m, 2 H), 1.43 (m, 2 H), 1.33 (m, 4 H). Anal. ($C_{15}H_{20}N_4O_6$) C, H, N.

Example 10 (Scheme 2d). 2-[Bis(2-Chloroethyl)amino]-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (IIb-2) and 2-[bis(2-bromoethyl)amino]-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (IIb-7). 2-Chloro-3,5-dinitrobenzoic acid (**11**) (17 g, 79.3 mmol) was treated with $SOCl_2$ (60 ml) containing one drop of DMF under reflux for 2 h. Evaporation of reagent followed by azeotroping with benzene gave the crude acid chloride, which was dissolved in Me_2CO (120 mL), cooled in ice-bath and treated with 2-aminoethanol (7.9 g). After stirring for 20 min. the reaction mixture was acidified to pH 4-5 with 1 N HCl, most of the solvent was evaporated, and the residue was partitioned between water (50 mL) and EtOAc (100 mL). The heterogeneous mixture was stood for 2 h, and the resulting precipitate was collected and washed with EtOAc. The filtrate was separated, the aqueous phase was extracted with EtOAc, and the combined organic phases were washed with sat. $NaHCO_3$, 1 N HCl and brine respectively, then concentrated to 50 mL, to give a second crop. The combined yield of 2-chloro-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (**12**) was 6.5 g (35%): mp (EtOAc) 159-160 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.99 (d, J = 2.6 Hz, 1 H, H-4), 8.86 (m, 1 H,

CONH), 8.56 (d, J = 2.6 Hz, 1 H, H-6), 4.83 (m, 1 H, OH), 3.54 (m, 4 H). Anal. ($C_9H_8ClN_3O_6$) C, H, N.

A solution of **12** (2.24 g, 11.08 mmol) and Et_3N (8 mL) in *p*-dioxane (120 mL) was treated with N,N-bis(2-chloroethyl)amine hydrochloride (6.0 g, 33.0 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc to gave the crude product which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) gave **IIb-2** (2.56 g, 61%): mp (EtOAc/petroleum ether) 109-111°C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (d, J = 2.6 Hz, 1 H, H-4), 8.72 (m, 1 H, CONH), 8.34 (d, J = 2.6 Hz, 1 H, H-6), 4.83 (m, 1 H, OH), 3.72 (m, 4 H, 2xCH_2Cl), 3.55 (m, 2 H), 3.42 (m, 4 H, 2xCH_2N), 3.34 (m, 2 H); ^{13}C NMR δ 165.33, 145.77, 145.33, 140.96, 136.32, 127.46, 122.13, 59.13, 54.05, 42.10, 41.50. HRMS (FAB) [MH^+] Calcd. For $C_{13}H_{17}{}^{35}Cl_2N_4O_6$ *m/z* 395.0525. Found; 395.0525.

A solution of **IIb-2** (1.20 g, 3.04 mmol) and LiBr (5 g) in 3-methyl-2-butanone (20 mL) was heated under reflux for 6 h, then cooled and poured into water. Extraction with EtOAc gave a crude product (<95 % pure), that was re-treated with LiBr (5 g) in 3-methyl-2-butanone for a further 4 h, then worked up and chromatographed on silica gel, eluting with EtOAc/petroleum ether (from 1:1 to 1:0), to give **IIb-7** (1.39 g, 94.6%): mp (EtOAc/petroleum ether) 105-108 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.7 Hz, 1 H, H-4), 8.73 (m, 1 H, CONH), 8.34 (d, J = 2.7 Hz, 1 H, H-6), 4.83 (m, 1 H, OH), 3.59-3.29 (m, 12 H); ^{13}C NMR δ 165.31, 145.40, 145.30, 141.12, 136.46, 127.42, 122.10, 59.25, 53.92, 42.09, 29.96. HRMS (FAB) Calcd. For $C_{13}H_{17}{}^{79}Br_2N_4O_6$ [$M+H^+$] *m/z* 482.9515. Found; 482.9492. Anal. ($C_{13}H_{16}Br_2N_4O_6$) H, N, Br; C: found, 32.9; calculated 32.3%.

Example 11 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-*N*-(3-hydroxypropyl)-3,5-dinitrobenzamide (IIb-3) and 2-[bis(2-bromoethyl)amino]-*N*-(3-hydroxypropyl)-3,5-dinitrobenzamide (IIb-8). Reaction of the acid chloride of **11** (17 g) with 3-aminopropanol (7.5 g) in Me_2CO (120 mL) at 0 °C as described above, gave 2-chloro-*N*-(3-hydroxypropyl)-3,5-dinitrobenzamide **(13)** (5.06 g, 26%): mp (EtOAc/petroleum ether) 120-121 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.99 (d, J = 2.6 Hz, 1 H, H-4), 8.79 (m, 1 H, CONH), 8.51 (d, J = 2.6 Hz, 1 H,

H-6), 4.50 (m, 1 H, OH), 3.49 (m, 2 H), 3.32 (m, 2 H), 1.70 (m, 2 H). Anal. ($C_{10}H_{10}ClN_3O_6$) C, H, N.

A solution of **13** (1.39 g, 4.58 mmol) and Et_3N (4 mL) in *p*-dioxane (60 mL) was treated with N,N-bis(2-chloroethyl)amine hydrochloride (2.9 g, 16.0 mmol) at 50 C for 24 h. Workup as described above gave **IIb-3** (1.84 g, 100%): mp (EtOAc/petroleum ether) 89-91 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.7 Hz, 1 H, H-4), 8.71 (m, 1 H, CONH), 8.30 (d, J = 2.7 Hz, 1 H, H-6), 4.52 (m, 1 H, OH), 3.71 (m, 4 H, 2xCH_2Cl), 3.50 (m, 2 H), 3.42 (m, 4 H, 2xCH_2N), 3.32 (m, 2 H), 1.71 (m, 2 H); ^{13}C NMR δ 165.10, 145.71, 145.47, 141.01, 136.35, 127.31, 122.08, 58.39, 54.13, 41.50, 36.69, 31.76. HRMS (FAB) Calcd. For $C_{14}H_{19}{}^{35}Cl_2N_4O_6$ [M+H$^+$] *m/z* 409.0682. Found; 409.0678.

Treatment of **IIb-3** with LiBr in 3-methyl-2-butanone twice, as described above, gave **IIb-8** (74% yield): mp (EtOAc/petroleum ether) 89-94 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.7 Hz, 1 H, H-4), 8.72 (m, 1 H, CONH), 8.30 (d, J = 2.7 Hz, 1H, H-6), 3.77-3.44 (m, 12 H), 1.70 (m, 2 H); ^{13}C NMR δ 165.07, 145.54, 145.25, 141.16, 136.46, 127.28, 122.06, 58.39, 54.01, 36.69, 31.77, 29.92. HRMS (FAB) Calcd. For $C_{14}H_{19}{}^{79}Br_2N_4O_6$ [M+H$^+$] *m/z* 496.9671. Found; 496.9658.

Example 12 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-*N*-(4-hydroxybutyl)-3,5-dinitrobenzamide (IIb-4) and 2-[bis(2-bromoethyl)amino]-*N*-(4-hydroxybutyl)-3,5-dinitrobenzamide (IIb-9). Reaction of the acid chloride of **11** (2.65 g, 10 mmol) with 4-aminobutanol (1.9 g) as above, followed by acidification to pH 4-5 with 1 N HCl and evaporation of most of the solvent gave a residue. This was partitioned between water (50 mL) and EtOAc (100 mL). The aqueous phase was extracted with EtOAc, and the combined organic phase were washed with sat. $NaHCO_3$, 1 N HCl and brine respectively, then concentrated to give 2-chloro-*N*-(4-hydroxybutyl)-3,5-dinitrobenzamide (**14**) 1.11 g (35%): mp (EtOAc) 121-124 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.98 (d, J = 2.7 Hz, 1H), 8.79 (m, 1 H), 8.52 (d, J= 2.7 Hz, 1H), 4.43 (m, 1 H), 3.43 (m, 2H), 3.26 (m, 2 H), 1.54 (m, 4 H); ^{13}C NMR δ 162.6, 148.4, 145.9, 140.4, 128.2, 125.8, 120.4, 60.2, 39.1, 29.8, 25.3. Anal. ($C_{11}H_{12}ClN_3O_6$) C, H, N.

A solution of **14** (0.75 g, 2.3 mmol) and Et_3N (2 mL) in *p*-dioxane (30 mL) was treated with N,N-bis(2-chloroethyl)amine hydrochloride (1.5 g, 8.0 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc gave the crude product which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) gave **IIb-4** (0.99 g, 100%) as yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.71 (d, J = 2.8 Hz, 1 H), 8.69 (m, 1 H), 8.27 (d, J = 2.8 Hz, 1 H), 4.37 (m, 1 H), 3.70 (m, 4 H), 3.38 (m, 6 H), 3.25 (m, 2 H), 1.56 (m, 2 H), 1.47 (m, 2 H); ^{13}C NMR δ 165.0, 145.7, 145.5, 141.0, 136.4, 127.2, 122.0, 60.2, 54.2, 41.5, 39.2, 29.8, 25.2. HRMS (FAB) Calcd. For $C_{15}H_{21}{}^{35}Cl_2N_4O_6$ [M+H$^+$] *m/z* 423.0838. Found; 423.0847.

A solution of **IIb-4** (0.96 g, 3.04 mmol) and LiBr (5 g) in 3-methyl-2-butanone (15 mL) was heated under reflux for 6 h, then cooled and poured into water. Extraction with EtOAc gave a crude product (<95 % pure), that was re-treated with LiBr (5 g) in 3-methyl-2-butanone for a further 4 h, then worked up and chromatographed on silica gel, eluting with EtOAc/petroleum ether (from 1:1 to 3:1) give **IIb-9** (1.01 g, 87%) as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.8 Hz, 1 H), 8.72 (m, 1 H), 8.28 (d, J = 2.8 Hz, 1 H), 3.60-3.26 (m, 12 H), 1.58 (m, 2 H), 1.49 (m, 2 H); ^{13}C NMR δ 165.0, 145.6, 145.2, 141.2, 136.5, 127.2, 122.0, 60.2, 54.1, 39.2, 29.9, 29.8, 25.2. HRMS (FAB) Calcd. For $C_{15}H_{21}{}^{79}Br_2N_4O_6$ [M+H$^+$] *m/z* 510.9828. Found; 510.9832.

Example 13 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-*N*-(5-hydroxypentyl)-3,5-dinitrobenzamide (IIb-5) and 2-[bis(2-bromoethyl)amino]-*N*-(5-hydroxypentyl)-3,5-dinitrobenzamide (IIb-10). Similar reaction of the acid chloride of **11** with 5-aminopentanol as above gave 2-chloro-*N*-(5-hydroxypentyl)-3,5-dinitrobenzamide (**15**), 1.3 g (39%), mp (EtOAc) 105-108 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.98 (d, J = 2.7 Hz, 1H), 8.79 (m, 1 H), 8.50 (d, J = 2.7 Hz, 1 H), 4.35 (m, 1 H), 3.39 (m, 2 H), 3.26 (m, 2 H), 1.54 (m, 2 H), 1.44 (m, 2 H), 1.36 (m, 2 H); ^{13}C NMR δ 162.7, 148.4, 145.9, 140.4, 128.2, 125.8, 120.4, 60.5, 39.1, 32.0, 28.4, 22.8. Anal. ($C_{12}H_{14}ClN_3O_6$) C, H, N.

A solution of **15** (0.63 g, 2.3 mmol) and Et_3N (2 mL) in *p*-dioxane (30 mL) was treated with N,N-bis(2-chloroethyl)amine hydrochloride (1.5 g, 8.0 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc to gave the crude product which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) gave **IIb-5** (0.82 g, 100%) as yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (d, J = 2.8 Hz, 1 H), 8.69 (m, 1 H), 8.28 (d, J = 2.8 Hz, 1 H), 4.32 (m, 1 H), 3.70 (m, 4 H), 3.40 (m, 6 H), 3.25 (m, 2 H), 1.55 (m, 2 H), 1.47 (m, 2 H), 1.37 (m, 2 H); ^{13}C NMR δ 165.0, 145.7, 145.5, 141.0, 136.4, 127.2, 122.0, 60.5, 54.2, 41.5, 39.3, 32.0, 28.3, 22.9. HRMS (FAB) Calcd. For $C_{16}H_{23}{}^{35}Cl_2N_4O_6$ [M+H$^+$] *m/z* 437.0995. Found; 437.0991.

Similar reaction of **IIb-5** (1.3 g) with LiBr gave **IIb-10** (1.35 g, 86%) as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.8 Hz, 1 H), 8.71 (m, 1 H), 8.28 (d, J = 2.8 Hz, 1 H), 3.60-3.26 (m, 12 H), 1.55 (m, 2 H), 1.48 (m, 2 H), 1.37 (m, 2 H); ^{13}C NMR δ 165.0, 145.6, 145.2, 141.2, 136.5, 127.2, 122.0, 60.5, 54.1, 39.3, 32.0, 29.8, 28.4, 22.9. HRMS (FAB) Calcd. For $C_{16}H_{23}{}^{79}Br_2N_4O_6$ [M+H$^+$] *m/z* 524.9984. Found; 524.9975.

Example 14 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (IIb-6) and 2-[bis(2-bromoethyl)amino]-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (IIb-11). Similar reaction of the acid chloride of **11** with 6-aminohexanol as above gave 2-chloro-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (**16**), 0.9 g (26%), mp (EtOAc) 88-91 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.98 (d, J = 2.7 Hz, 1H), 8.78 (m, 1 H), 8.49 (d, J = 2.7 Hz, 1 H), 4.32 (m, 1 H), 3.39 (m, 2H), 3.26 (m, 2 H), 1.54 (m, 2 H), 1.44 (m, 2 H), 1.34 (m, 4 H); ^{13}C NMR δ 162.7, 148.4, 145.9, 140.4, 128.2, 125.8, 120.4, 60.5, 39.1, 32.3, 28.6, 26.2, 25.1. Anal. ($C_{13}H_{16}ClN_3O_6$) C, H, N.

A solution of **16** (0.67 g, 2.5 mmol) and Et_3N (2 mL) in *p*-dioxane (30 mL) was treated with N,N-bis(2-chloroethyl)amine hydrochloride (1.5 g, 8.0 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc to gave the crude product which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) gave **IIb-6** (0.87 g, 100%) as yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (d, J = 2.8 Hz, 1 H), 8.70 (m, 1 H), 8.28 (d, J = 2.8 Hz, 1 H), 4.31 (m, 1 H), 3.70 (m, 4 H), 3.38 (m, 6 H), 3.25 (m, 2 H), 1.54 (m, 2 H),

1.40 (m, 2 H), 1.32 (m, 4 H); ^{13}C NMR δ 165.0, 145.7, 145.6, 141.0, 136.4, 127.2, 122.0, 60.5, 54.2, 41.5, 39.2, 32.3, 28.5, 26.3, 25.1. HRMS (FAB) Calcd. For $C_{17}H_{25}{}^{35}Cl_2N_4O_6$ [M+H$^+$] *m/z* 451.1151. Found; 451.1154.

Similar reaction of **IIb-6** (0.97 g) with LiBr gave **IIb-11** (0.955 g, 81%) as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.8 Hz, 1 H), 8.70 (m, 1 H), 8.28 (d, J = 2.8 Hz, 1 H), 3.60-3.26 (m, 12 H), 1.54 (m, 2 H), 1.43 (m, 2 H), 1.32 (m, 4 H); ^{13}C NMR δ 165.0, 145.6, 145.2, 141.2, 136.5, 127.2, 122.0, 60.6, 54.1, 39.2, 32.4, 29.9, 28.5, 26.3, 25.1. HRMS (FAB) Calcd. For $C_{17}H_{25}{}^{79}Br_2N_4O_6$ [M+H$^+$] *m/z* 539.0141. Found; 539.0135.

Example 15 (Scheme 2e). 2-[Bis(2-bromopropyl)amino]-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (IIb-7a). Reaction of 2-chloro-3,5-dinitro-N-[2-(tetrahydro-2H-pyran-2-yloxy)ethyl]benzamide (**17**) (1.02 g)[For method of preparation see co-pending NZ Application No. 521851] with diisopropanolamine (0.8 g) as above gave 2-[bis(2-hydroxypropanyl)amino]-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]benzamide (**18**) (1.29 g, 100%): as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 9.22 (br, 1H), 8.66 (d, J = 2.8 Hz, 1H), 8.29 (d, J= 2.8 Hz, 1H), 4.99 (m, 1 H), 4.85 (br, 1 H), 4.62 (br, 1 H), 3.94 (m, 2 H), 3.77 (m, 2 H), 3.53 (m, 4H), 3.26 (m, 2 H), 1.48 (m, 10 H), 0.98 (m, 6 H); ^{13}C NMR δ 166.5, 147.8, 142.4, 138.2, 132.6, 128.8, 123.8, 98.1, 64.8, 63.5, 61.5, 60.1, 30.1, 25.0, 20.5, 20.2, 19.1. HRMS (FAB) Calcd. For $C_{20}H_{31}N_4O_9$ [M+H$^+$] *m/z* 471.2091. Found; 471.2089.

Reaction of **18** with MsCl as above gave 2-[{2-[(methylsulfonyl)oxy]propanyl}-4,6-dinitro-6-({[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]amino}carbonyl)anilino]ethyl methanesulfonate (**19**) (2.52 g, 100%): as a yellow foam; which was used directly for the next step.

A solution of **19** (2.52 g, 4.03 mmol) in THF (150 mL) was treated with 1 N HCl (100 mL), and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized with satd. $NaHCO_3$, and extracted with EtOAc (3x80 mL). The combined organic phases were washed with brine and dried, the solvent was evaporated, and the residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH(100:1), to give 3-(2-{[(2-hydroxyethyl)amino]carbonyl}{3-[(methylsulfonyl)oxy]butyl}-4,6-dinitroanilino)-1-

methylpropyl methanesulfonate (**20**) (0.80 g, 37%): as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.94 (m, 1 H), 8.72 (m, 1 H), 8.35 (m, 1 H), 4.92 (m, 2 H), 3.56 (m, 2 H), 3.30 (m, 6 H), 3.16 (s, 6 H), 1.32 (m, 6 H); ^{13}C NMR δ 165.9, 145.8, 143.4, 139.4, 133.6, 128.0, 123.1, 76.3, 59.2, 57.3, 42.2, 37.7, 18.6. HRMS (FAB) Calcd. For $C_{17}H_{27}N_4O_{12}S_2$ [M+H$^+$] *m/z* 543.1067. Found; 543.1074.

Treatment of **20** (0.52 g, 0.96 mmol) with LiBr (0.5 g, 5.2 mmol) in EtOAc (50 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave **II-7a** (0.31 g, 62%): as yellow solid: mp (EtOAc/petroleum ether) 127-130 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.91 (m, 1H, CONH), 8.70 (d, J= 2.8 Hz, 1H, H-4), 8.32 (d, J=2.8 Hz, 1H, H-6), 4.80 (m, 1 H), 4.42 (m, 2 H), 3.55 (m, 4 H), 1.62 (m, 6 H); ^{13}C NMR δ 165.8, 144.8, 143.5, 139.6, 133.6, 128.0, 122.9, 60.6, 59.2, 47.9, 42.2, 23.4. Anal. ($C_{15}H_{20}Br_2N_4O_6$) C, H, N.

Example 16 (Scheme 2f). 2-((2-Bromoethyl)-2-{[(2-hydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate (IIb-13). A solution of **13** (1.22 g, 4.0 mmol) in 50 mL of CH_2Cl_2 was cooled in an ice-bath, and 3,4-dihydro-2*H*-pyran (1.0 mL) and p-toluenesulfonic acid (0.1 g) were added. The reaction mixture was stirred for 2 h, then concentrated under reduced pressure. Chromatography of the residue on silica gel, eluting with EtOAc/petroleum ether (from 1:2 to 2:1), gave 2-chloro-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]benzamide (**21**) (1.45 g, 94%): as a pale yellow oil; ^{1}H NMR [$(CD_3)_2SO$] δ 8.99 (d, J = 2.7 Hz, 1 H, H-4), 8.81 (m, 1 H, CONH), 8.51 (d, J = 2.7 Hz, 1 H, H-6), 4.57 (m, 1 H), 3.72 (m, 2 H), 3.46-3.25 (m, 4 H), 1.82-1.44 (m, 8 H). ^{13}C NMR δ 162.7, 148.4, 145.9, 140.3, 128.2, 125.8, 120.5, 98.0, 64.2, 61.3, 36.5, 30.2, 28.9, 24.9, 19.1. HRMS (FAB) Calcd. For $C_{15}H_{19}{}^{35}ClN_3O_7$ [M+H$^+$] *m/z* 388.0912. Found; 388.0915.

Reaction of **21** (1.45 g, 3.75 mmol) with diethanolamine (1.67 g) as above gave 2-[bis(2-hydroxyethyl)amino]-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]benzamide (**22**) (1.62 g, 95%): as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.96 (m, 1H, CONH), 8.66 (d, J= 2.8 Hz, 1H, H-4), 8.31 (d, J=2.8 Hz, 1H, H-6), 4.95 (m, 2H), 4.56 (m, 1H), 3.79-3.16 (m, 14H),

1.80-1.45 (m, 8 H); ^{13}C NMR δ 166.2, 148.1, 143.6, 139.3, 133.8, 128.9, 123.8, 98.5, 64.8, 61.7, 58.5, 54.6, 37.3, 30.6, 29.2, 25.4, 19.6. HRMS (FAB) Calcd. For $C_{19}H_{29}N_4O_6$ [M+H$^+$] *m/z* 457.1935. Found; 457.1939.

Reaction of **22** (1.62 g, 3.55 mmol) with MsCl (2 mL) as above gave 2-[{2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitro-6-({[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]-amino}carbonyl)anilino]ethyl methanesulfonate (**23**) (2.17 g, 100%): as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.71 (d, J = 2.8 Hz, 1H), 8.71 (m, 1H), 8.31 (d, J= 2.8 Hz, 1H), 4.26 (m, 4 H), 3.71-3.37 (m, 10 H), 3.13 (s, 6 H), 3.10 (m, 2 H), 1.82-1.43 (m, 8 H); ^{13}C NMR δ 165.1, 146.3, 145.4, 140.9, 135.9, 127.4, 122.2, 98.0, 67.2, 64.3, 51.4, 45.7, 36.5, 30.2, 28.7, 24.9, 19.1, 8.5. HRMS (FAB) Calcd. For $C_{21}H_{33}N_4O_{13}S_2$ [M+H$^+$] *m/z* 613.1486. Found; 613.1481.

A solution of **23** (2.95 g, 3.55 mmol) in THF (120 mL) was treated with 1 N HCl (80 mL), and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized with satd. $NaHCO_3$, and extracted with EtOAc (3x80 mL). The combined organic phases were washed with brine and dried, the solvent was evaporated, and the residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH(100:1), to give **24** (1.4 g, 75%): as a yellow solid: mp (EtOAc/petroleum ether) 130-133 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.8 Hz, 1H), 8.72 (m, 1H), 8.32 (d, J= 2.8 Hz, 1H), 4.29 (m, 4 H), 3.47 (m, 8 H), 3.14 (s, 6 H), 1.71 (m, 2 H); ^{13}C NMR δ 165.2, 146.3, 145.3, 140.8, 135.9, 127.5, 122.3, 67.3, 58.4, 51.4, 36.8, 36.5, 31.7. Anal. ($C_{16}H_{24}N_4O_{12}S_2$) C, H, N.

Treatment of **24** (0.25 g, 0.45 mmol) with LiBr (53 mg, 0.7 mmol) in EtOAc (50 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave **II-13** (0.16 g, 66%): as yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J = 2.8 Hz, 1H), 8.73 (m, 1H), 8.31 (d, J= 2.8 Hz, 1H), 4.28 (m, 2 H), 3.65-3.44 (m, 10 H), 3.13 (s, 3 H), 1.70 (m, 2 H); ^{13}C NMR δ 165.1, 145.7, 145.4, 141.0, 136.2, 127.3, 122.1, 67.5, 58.4, 51.1, 36.7, 36.5, 31.7, 29.6. HRMS (FAB) Calcd. For $C_{15}H_{22}{}^{79}BrN_4O_9S$ [M+H$^+$] *m/z* 513.0291. Found; 513.0281.

Example 17 (Scheme 2g). 2-[Bis(2-iodoethyl)amino]-*N*-(3-hydroxyethyl)-3,5-dinitrobenzamide (IIb-14) and 2-((2-iodoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate (IIb-15). Treatment of **25** [For method of preparation see co-pending NZ Application No. 521851]) (6.7 g, 13.0 mmol) with NaI (2.9 g, 20 mmol) in EtOAc (200 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave **IIb-14** (3.3 g, 44%) as a yellow solid: mp (EtOAc/petroleum ether) 129-131 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.72 (d, J = 2.8 Hz, 1 H, H-4), 8.70 (m, 1 H, CONH), 8.32 (d, J = 2.8 Hz, 1 H, H-6), 4.80 (m, 1 H), 3.55 (m, 2 H), 3.43 (m, 4 H), 3.31 (m, 6 H); ^{13}C NMR δ 165.3, 145.2, 144.7, 141.0, 136.3, 127.3, 122.0, 59.3, 54.7, 42.1, 2.94. Anal ($C_{13}H_{16}N_4I_2O_6$) C, H, N.

Later eluates gave **IIb-15** (1.35 g, 19%) as a yellow foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (d, J= 2.8 Hz, 1H, H-4), 8.74 (m, 1H, CONH), 8.34 (d, J=2.8 Hz, 1 H, H-6), 4.28 (m, 2 H), 3.56 (m, 2 H), 3.43 (m, 2 H), 3.31 (m, 6 H), 3.13 (s, 3 H); ^{13}C NMR δ 165.3, 145.5, 145.2, 140.8, 136.1, 127.4, 122.1, 67.5, 59.2, 55.4, 50.6, 42.1, 36.5, 2.6. HRMS (FAB) Calcd. For $C_{14}H_{20}IN_4O_9S$ [M+H$^+$] *m/z* 546.9996. Found; 546.9997.

Example 18 (Scheme 2h). 3-[Bis(2-bromoethyl)amino]-*N*-(2-hydroxyethyl)-2,6-dinitrobenzamide (IIc-7) and 2-((2-bromoethyl)-3-{[(2-hydroxyethyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate (IIc-12). Treatment of 3-(3-{[(2-hydroxyethyl)amino]carbonyl}{3-[(methylsulfonyl)oxy]butyl}-2,4-dinitroanilino)-1-methylpropyl methanesulfonate (**26**) [for method of preparation see NZ Application No. 521851] (310 mg, 0.6 mmol) in EtOAc (50 mL) with LiBr (78 mg, 0.9 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave **IIC-7** (70 mg, 25%) as a foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.80 (m, 1H, CONH), 8.24 (d, J= 9.4 Hz, 1H), 7.63 (d, J=9.4 Hz, 1H), 4.66 (m, 1 H), 3.70 (m, 4 H), 3.60 (m, 4 H), 3.45 (m, 2 H), 3.22 (m, 2 H); ^{13}C NMR δ 161.4, 145.8, 140.2, 137.5, 129.2, 127.6, 122.6, 59.0, 52.6, 41.7, 30.0.. HRMS (FAB) Calcd. For $C_{13}H_{17}{}^{79}Br_2N_4O_6$ [M+H$^+$] *m/z* 482.9515. Found; 482.9508.

Further elution with EtOAc/MeOH (50:2) gave **IIc-12** (118 mg, 39%): mp. 94-97 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.80 (m, 1H, CONH), 8.25 (d, J= 9.4 Hz, 1H), 7.64 (d, J=9.4 Hz, 1H),

4.67 (m, 1 H), 4.27 (m, 2 H), 3.63 (m, 4 H), 3.57 (m, 2 H), 3.45 (m, 2 H), 3.26 (m, 2 H), 3.15 (s, 3 H); ^{13}C NMR δ 161.4, 146.2, 140.5, 137.7, 129.2, 127.5, 122.9, 66.8, 59.0, 50.0, 41.7, 36.6, 29.9. Anal. ($C_{14}H_{19}BrN_4O_9S$) C, H, N.

Example 19 (Scheme 2h). 3-[Bis(2-bromoethyl)amino]-*N*-(3-hydroxypropyl)-2,6-dinitrobenzamide (IIc-8) and 2-((2-bromoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate (IIc-13). Treatment of 3-(3-{[(3-hydroxypropyl)amino]carbonyl}{3-[(methylsulfonyl)oxy]butyl}-2,4-dinitroanilino)-1-methylpropyl methanesulfonate (**27**) [for method of preparation see co-pending NZ Application No. 521851] (716 mg, 1.36 mmol) in EtOAc (200 mL) with LiBr (175 mg, 2.0 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave **IIc-8** (289 mg, 42%) as a foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.75 (t, J = 5.8 Hz, 1 H, CONH), 8.23 (d, J = 9.4 Hz, 1 H, H-5), 7.62 (d, J = 9.4 Hz, 1 H, H-6), 4.47 (m, 1 H, CHO*H*), 3.68 (m, 4 H), 3.57 (m, 4 H), 3.43 (m, 2 H), 3.20 (m, 2 H), 1.60 (m, 2 H); ^{13}C NMR δ 161.20, 146.90, 140.20, 137.53, 129.36, 127.69, 122.56, 58.29, 52.64, 36.42, 31.61, 30.13. HRMS (FAB) Calcd. For $C_{14}H_{19}{}^{79}Br_2N_4O_6$ [M+H$^+$] *m/z* 496.9671. Found: 496.9667.

Further elution with EtOAc/MeOH (50:2) gave **IIc-13** (270 mg, 39%): mp. 115-117 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.75 (t, J = 5.8 Hz, 1 H, CONH), 8.24 (d, J = 9.4 Hz, 1 H, H-5), 7.64 (d, J = 9.4 Hz, 1 H, H-6), 4.43 (m, 1 H, CHO*H*), 4.27 (m, 2 H, C*H*$_2$OMs), 3.66 (m, 4 H, 2xCH$_2$N), 3.59 (m, 2 H), 3.44 (m, 2 H), 3.22 (m, 2 H), 3.15 (s, 3 H, CH_3SO_3), 1.60 (m, 2 H); ^{13}C NMR δ 161.08, 146.19, 140.47, 137.69, 129.24, 127.59, 122.91, 66.83, 58.22, 52.87, 50.00, 36.57, 36.37, 31.58, 29.95. Anal. ($C_{15}H_{21}BrN_4O_9S$) C, H, N.

Example 20 (Scheme 2h). 3-[Bis(2-bromoethyl)amino]-*N*-(4-hydroxybutyl)-2,6-dinitrobenzamide (IIc-9) and 2-((2-bromoethyl)-3-{[(4-hydroxybutyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate (IIc-14). Treatment of 3-(3-{[(4-hydroxybutyl)amino]carbonyl}{3-[(methylsulfonyl)oxy]butyl}-2,4-dinitroanilino)-1-methylpropyl methanesulfonate (**28**) [for method of preparation see NZ Application No. 521851] (500 mg, 0.92 mmol) in EtOAc (100 mL) with LiBr (110 mg, 1.4 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0)

gave **IIc-9** (100 mg, 21%) as a foam; ^{1}H NMR [$(CD_3)_2SO$] δ 8.73 (m, 1H, CONH), 8.25 (d, J= 9.4 Hz, 1H), 7.63 (d, J=9.4 Hz, 1H), 4.38 (m, 1 H), 3.69 (m, 4 H), 3.57 (m, 4 H), 3.40 (m, 2 H), 3.14 (m, 2 H), 1.47 (m, 4 H); ^{13}C NMR δ 161.0, 145.8, 140.2, 137.6, 129.3, 127.6, 122.6, 60.2, 52.6, 30.0, 29.6, 24.8. HRMS (FAB) Calcd. For $C_{15}H_{20}{}^{79}Br_2N_4O_6$ [M+H$^+$] *m/z* 510.9828. Found; 510.9819.

Further elution with EtOAc/MeOH (50:2) gave **IIc-14** (117 mg, 30%): mp. 114-117 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.74 (m, 1 H, CONH), 8.25 (d, J = 9.4 Hz, 1 H), 7.65 (d, J =9.4 Hz, 1 H), 4.37 (m, 1 H), 4.27 (m, 2 H), 3.65 (m, 4 H), 3.57 (m, 2 H), 3.35 (m, 2 H), 3.16 (m, 2 H), 3.15 (s, 3 H), 1.47 (m, 4 H); ^{13}C NMR δ 160.0, 146.1, 140.6, 137.8, 129.2, 127.5, 122.9, 66.8, 60.2, 52.9, 50.0, 36.6, 29.9, 29.6, 24.9. Anal. ($C_{16}H_{23}BrN_4O_9S$) C, H, N.

Preparation of Phosphates (Scheme 3)

Example 21. 2-[[2-[Bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate (Ib-7P). A solution of alcohol **IIb-7** (2.58 g, 5.33 mmol) and di-*tert*-butyl diethylphosphoramidite (93%, 2.07 mL, 6.93 mmol) in dry DMF (20 mL) under N_2 was treated with 1*H*-tetrazole (3 wt. % in CH_3CH, 55.1 mL, 18.7 mmol) and stirred at 20 °C for 1.5 h. The reaction mixture was then cooled to –50 °C and a solution of 3-chloroperoxybenzoic acid (55%, 2.68 g, 8.54 mmol) was rapidly added such that the temperature was kept below –5 °C. The reaction mixture was warmed to room temperature and diluted with CH_2Cl_2 (150 mL). The solution was washed with 5% aqueous $Na_2S_2O_5$ (2 x 50 mL), 10% aqueous $NaHCO_3$ (2 x 50 mL), water (2 x 50 mL), dried, concentrated under reduced pressure below 30 °C and the residue was shaken with i-Pr_2O/hexane and refrigerated. The resulting solid was purified by chromatography on silica gel, eluting with CH_2Cl_2/EtOAc, followed by recrystallisation from CH_2Cl_2/hexane (below 40 °C) to give di-*tert*-butyl 2-[[2-[bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl phosphate **(Ib-7E)**(2.59 g, 72%) as an unstable yellow solid: mp 99-101 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 8.93 (t, *J* = 5.6 Hz, 1 H), 8.76 (d, *J* = 2.8 Hz, 1 H), 8.33 (d, *J* = 2.8 Hz, 1 H), 4.01 (g, *J* = 6.1 Hz, 2 H), 3.62-3.42 (m, 10 H), 1.43 (s, 18 H). HRMS (FAB) calcd for $C_{21}H_{34}{}^{79}Br_2N_4O_9P$ (MH$^+$) *m/z* 675.0430

found 675.0398; calcd for $C_{21}H_{34}{}^{79}Br^{81}BrN_4O_9P$ (MH^+) *m/z* 677.0410, found 677.0397; calcd for $C_{21}H_{34}{}^{81}Br_2N_4O_9P$ (MH^+) *m/z* 679.0389, found 679.0398. Anal. ($C_{21}H_{33}Br_2N_4O_9P$).

A solution of **Ib-7E** (2.80 g, 4.14 mmol) and TFA (15 mL) in dry CH_2Cl_2 (15 mL) was stirred at 20 °C for 1 h, then concentrated under reduced pressure. Residual TFA was removed azeotropically with CH_3CN (2 x) and the resulting residue was dissolved in EtOAc. Addition of excess hexane precipitated a semisolid which was dried under high vacuum at 20 °C to give **Ib-7P** (98%) as a yellow foam. ^1H NMR [$(CD_3)_2SO$] δ 8.93 (t, J = 5.6 Hz, 1 H), 8.75 (d, J = 2.8 Hz, 1 H), 8.36 (d, J = 2.8 Hz, 1 H), 3.97 (q, J = 6.3 Hz, 2 H), 3.62-3.43 (m, 10 H). HRMS (FAB) calcd for $C_{13}H_{18}{}^{79}Br_2N_4O_9P$ (MH^+) *m/z* 562.9178, found 562.9171; calcd for $C_{13}H_{18}{}^{79}Br^{81}BrN_4O_9P$ (MH^+) *m/z* 564.9158, found 564.9152; calcd for $C_{13}H_{18}{}^{81}Br_2N_4O_9P$. ($MH^+$) *m/z* 566.9137, found 566.9121. Treatment of diacid **Ib-7P** with $NaHCO_3$ (2.0 equiv.) gave the disodium salt.

Example 22. 3-[[5-[Bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate (Ia-3P). Similar phosphorylation of **IIa-3**, followed by chromatography of the product on silica gel and elution with CH_2Cl_2/EtOAc (2:3), gave di-*tert*-butyl 3-[[5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl phosphate **(Ia-3E)** (76%) as a yellow solid: mp (EtOAc/i-Pr_2O/hexane) 120-121°C (dec); ^1H NMR [$(CD_3)_2SO$] δ 8.70 (t, J = 5.6 Hz, 1 H), 8.55 (s, 1 H), 7.45 (s, 1 H), 3.96 (q, J = 6.7 Hz, 2 H), 3.82 (t, J = 5.8 Hz, 4 H), 3.69 (t, J = 5.8 Hz, 4 H), 3.34 (after D_2O exchange, t, J = 6.8 Hz, 2 H), 1.86 (pent, J = 6.6 Hz, 2 H), 1.42 (s, 18 H). Anal. ($C_{22}H_{35}Cl_2N_4O_9P$) C, H, N.

Similar treatment of ester **Ia-3E** with TFA gave diacid **Ia-3P** (99%) as a hygroscopic yellow solid. ^1H NMR [$(CD_3)_2SO$] δ 8.71 (t, J = 5.6 Hz, 1 H), 8.54 (s, 1 H), 7.45 (s, 1 H), 3.92 (q, J = 6.7 Hz, 2 H), 3.82 (t, J = 5.8 Hz, 4 H), 3.69 (t, J = 5.8 Hz, 4 H), 3.31 (q, J = 6.5 Hz, 2 H), 1.84 (pent, J = 6.6 Hz, 2 H). HRMS (FAB) Calcd. for $C_{14}H_{20}{}^{35}Cl_2N_4O_9P$ $[M+H]^+$ *m/z* 489.0345; found 489.0344. Calcd. for $C_{14}H_{20}{}^{35}Cl^{37}ClN_4O_9P$ $[M+H]^+$ *m/z* 491.0316; found 491.0317. Calcd. for $C_{14}H_{20}{}^{37}Cl_2N_4O_9P$ $[M+H]^+$ *m/z* 493.0286; found 493.0312. Treatment of diacid **I-3P** with $NaHCO_3$ (2:0 equiv) gave the disodium salt.

Example 23. 3-[[5-[Bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate (Ia-8P). Similar phosphorylation of **IIa-8**, followed by chromatography of the product on silica gel and elution with CH_2Cl_2/EtOAc (1:1), gave di-*tert*-butyl 3-[[5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl phosphate **(Ia-8E)** (66%) as a yellow solid: mp (EtOAc/i-Pr_2O/hexane) 110-111°C (dec). ^{1}H NMR ($(CD_3)_2SO$) δ 8.70 (t, J = 5.6 Hz, 1 H), 8.55 (s, 1 H), 7.44 (s, 1 H), 3.96 (q, J = 6.7 Hz, 2 H), 3.79-3.63 (m, 84 H), 3.35 (after D_2O exchange, t, J = 6.8 Hz, 2 H), 1.86 (pent, J = 6.6 Hz, 2 H), 1.42 (s, 18 H). Anal. ($C_{22}H_{35}Br_2N_4O_9P$) C, H, N.

Similar treatment of ester **Ia-8E** with TFA gave diacid **Ia-8P** (99%) as a hygroscopic yellow solid. ^{1}H NMR [$(CD_3)_2SO$] δ 8.71 (t, J = 5.6 Hz, 1 H), 8.55 (s, 1 H), 7.43 (s, 1 H), 3.93 (q, J = 6.7 Hz, 2 H), 3.79–3.63 (m, 8 H), 3.31(q, J = 6.5 Hz, 2 H), 1.85 (pent, J = 6.6 Hz, 2 H). HRMS (FAB) calcd for $C_{14}H_{20}{}^{79}Br_2N_4O_9P$ (MH^+) *m/z* 576.9335, found 576.9314; calcd for $C_{14}H_{20}{}^{79}Br^{81}BrN_4O_9P$ (MH^+) *m/z* 578.9314, found 578.9305; calcd for $C_{14}H_{20}{}^{81}Br_2N_4O_9P$ (MH^+) *m/z* 580.9294, found 580.9297. Treatment of diacid **Ia-8P** with $NaHCO_3$ (2.0 equiv.) gave the disodium salt.

Example 24. 2-[[2-[Bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate (Ib-2P). Similar phosphorylation of **IIb-2**, followed by chromatography of the product on silica gel and elution with CH_2Cl_2/EtOAc (13:7), gave di-*tert*-butyl 2-[[2-[bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl phosphate **(Ib-2E)** (72%) as a yellow solid: mp (EtOAc/i-Pr_2O/hexane) 107-108 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 8.90 (t, J = 5.6 Hz, 1 H), 8.75 (d, J = 2.8 Hz, 1 H), 8.33 (d, J = 2.8 Hz, 1 H), 4.01 (q, J = 6.1 Hz, 2 H), 3.72 (t, J = 6.8 Hz, 4 H), 3.53 (q, J = 5.5 Hz, 2 H), 3.43 (t, J = 6.8 Hz, 4 H), 1.43 (s, 18 H). Anal. ($C_{21}H_{33}Cl_2N_4O_9P$) C, H, N, P. CRL 11363.

Similar treatment of ester **Ib-2E** with TFA gave diacid **Ib-2P** (98%) as a yellow foam. ^{1}H NMR [$(CD_3)_2SO$] δ 8.89 (t, J = 5.6 Hz, 1 H), 8.74 (d, J = 2.8 Hz, 1 H), 8.36 (d, J = 2.8 Hz, 1 H), 3.98 (q, J = 6.2 Hz, 2 H), 3.72 (t, J = 6.7 Hz, 4 H), 3.51 (q, J = 5.6 Hz, 2 H), 3.43 (t, J = 6.7 Hz, 4 H). HRMS (FAB) Calcd. for $C_{13}H_{18}{}^{35}Cl_2N_2O_9P$ $[M+H]^+$ *m/z* 475.0189; found 475.0189. Calcd. for $C_{13}H_{18}{}^{35}Cl^{37}ClN_2O_9P$ $[M+H]^+$ *m/z* 477.0159; found 477.0167. Calcd.

for $C_{13}H_{18}{}^{35}Cl_2N_2O_9P$ $[M+H]^+$ *m/z* 479.0130; found 479.0160. Treatment of diacid **Ib-2P** with $NaHCO_3$ (1.0 equiv.) gave the monosodium salt.

Example 25. 2-({2-[Bis(2-bromopropyl)amino]-3,5-dinitrobenzoyl}amino)ethyl dihydrogen phosphate (Ib-7aP). Similar phosphorylation of alcohol **IIb-7a** (0.67 g, 1.3 mmol) with di-*tert*-butyldiethylphosphoramidite (93%, 489 mg, 2.0 mmol), followed by flash column chromatography on silica gel, eluting with EtOAc/petroleum ether (1:1) gave **Ib-7aE** as a yellow solid (0.74 g, 81%): mp (EtOAc/petroleum ether) 121-123 °C; ^{1}H NMR $[(CD_3)_2SO]$ δ 9.09 (m, 1 H), 8.73 (m, 1 H), 8.32 (m, 1 H), 4.44 (m, 2 H), 4.00 (m, 2 H), 3.39 (m, 2 H), 3.60 (m, 4 H), 1.62 (m, 6 H), 1.44 (s, 18 H). ^{13}C NMR δ 165.9, 144.8, 143.6, 139.6, 133.2, 128.0, 123.1, 81.6, 64.0, 60.4, 39.9, 29.4, 23.5. Anal. ($C_{23}H_{37}Br_2N_4O_9P$) C, H, N.

Similar treatment of **Ib-7aE** (100 mg) with TFA (6 mL), followed by crystallization from CH_2Cl_2/EtOAc, gave **Ib-7aP** as a yellow solid (70 mg, 85%): mp 157-161 °C; ^{1}H NMR $[(CD_3)_2SO]$ δ 9.07 (m, 1 H), 8.72 (m, 1 H), 8.36 (m, 1 H), 4.43 (m, 2 H), 4.00 (m, 2 H), 3.52 (m, 6 H), 1.62 (m, 6 H). ^{13}C NMR δ 165.9, 144.8, 143.6, 139.7, 133.4, 128.1, 123.1, 63.2, 60.4, 47.9, 39.9, 23.5. Anal. ($C_{15}H_{21}Br_2N_4O_9P$) C, H, N.

Example 26. 2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate (Ib-12P). Similar phosphorylation of **IIa-12**, followed by chromatography of the product on silica gel and elution with EtOAc/MeOH (19:1), gave 2-[(2-bromoethyl)-2-(6-*tert*-butoxy-8,8-dimethyl-6-oxido-5,7-dioxa-2-aza-6-phosphanon-1-anoyl)-4,6-dinitroanilino]ethyl methanesulfonate **(Ib-12E)** (66%) as a yellow foam. ^{1}H NMR $[(CD_3)_2SO]$ δ 8.94 (t, J = 5.6 Hz, 1 H), 8.75 (d, J = 2.8 Hz, 1 H), 8.34 (d, J = 2.8 Hz, 1 H), 4.28 (t, J = 5.4 Hz, 2 H), 4.02 (q, J = 6.2 Hz, 2 H), 3.62-3.43 (m, 8 H), 3.13 (s, 3 H), 1.43 (s, 18 H). HRMS (FAB) calcd for $C_{22}H_{37}{}^{79}BrN_4O_{12}PS$ $[M+H]^+$ *m/z* 693.1029; found 693.1010.

Similar treatment of ester **Ib-12E** with TFA gave diacid **Ib-12P** (98%) as a yellow foam. ^{1}H NMR $[(CD_3)_2SO]$ δ 8.92 (t, J = 5.6 Hz, 1 H), 8.74 (d, J = 2.8 Hz, 1 H), 8.37 (d, J = 2.8 Hz, 1 H), 4.28 (t, J = 5.4 Hz, 2 H), 3.98 (q, J = 6.0 Hz, 2 H), 3.58-3.40 (after D_2O exchange, m, 8

H), 3.13 (s, 2 H). HRMS (FAB) calcd for $C_{14}H_{21}{}^{79}BrN_4O_{12}PS$ $[M+H]^+$ *m/z* 578.9798; found 578.9784; calcd for $C_{14}H_{21}{}^{81}Br^{81}BrN_4O_{12}PS$ $[M+H]^+$ *m/z* 580.9777; found 580.9784. Treatment of diacid **Ib-12P** with $NaHCO_3$ (1.0 equiv) gave the monosodium salt.

Example 27. 2-[[2-[Bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate (Ib-14P). Similar phosphorylation of **Ib-14**, followed by chromatography of the product on silica gel and elution with CH_2Cl_2/EtOAc (3:1), gave di-*tert*-butyl 2-[[2-[bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl phosphate **(Ib-14E)** (67%) as a yellow solid: mp (CH_2Cl_2/i-Pr_2O/hexane) 108-110 °C (dec); ^{1}H NMR [$(CD_3)_2SO$] δ 8.91 (t, J = 5.6 Hz, 1 H), 8.74 (d, J = 2.8 Hz, 1 H), 8.30 (d, J = 2.8 Hz, 1 H), 4.01 (q, J = 6.3 Hz, 2 H), 3.53 (q, J = 5.7 Hz, 2 H), 3.45 (t, J 7.8 Hz, 4 H), 3.24 (after D_2O exchange, t, J = 7.6 Hz, 4 H), 1.44 (s, 18 H). Anal. ($C_{21}H_{33}I_2N_4O_9P$), C, H, N, P.

Similar treatment of ester **Ib-14E** with TFA gave diacid **Ib-14P** (97%) as a yellow foam. ^{1}H NMR [$(CD_3)_2SO$] δ 8.90 (t, J = 5.6 Hz, 1 H), 8.73 (d, J = 2.8 Hz, 1 H), 8.34 (d, J = 2.8 Hz, 1 H), 3.98 (q, J = 6.4 Hz, 2 H), 3.49 (after D_2O exchange t, J = 5.6 Hz, 2 H), 3.45 (t, J = 7.8 Hz, 4 H), 3.29 (t, J = 7.7 Hz, 4 H). HRMS (FAB) Calcd. for $C_{13}H_{18}I_2N_4O_9$ $[M+H]^+$ *m/z* 658.3911; found 658.3907. Treatment of diacid **Ib-14P** with $NaHCO_3$ (2.0 equiv.) gave the disodium salt.

Example 28. 2-[(2-Iodoethyl)-2,4-dinitro-6-({[2-(phosphonooxy)ethyl]amino}carbonyl)-anilino]ethyl methanesulfonate (Ib-15P). Similar phosphorylation of alcohol **IIb-15** (1.68 g, 3.1 mmol) with di-*tert*-butyldiethylphosphoramidite (93%, 1.15 g, 4.5 mmol), followed by flash column chromatography on silica gel, eluting with EtOAc/petroleum ether (1:1), and crystallization from EtOAc/petroleum ether, gave **Ib-15E** as a yellow solid (2.23 g, 97%): mp (EtOAc/petroleum ether) 109-111 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.98 (m, 1 H), 8.76 (d, J = 2.8 Hz, 1 H), 8.33 (d, J = 2.8 Hz, 1 H), 4.27 (m, 2 H), 4.00 (m, 2 H), 3.53 (m, 2 H), 3.46 (m, 4 H), 3.14 (s, 3 H), 1.43 (s, 18 H). ^{13}C NMR δ 165.5, 145.6, 145.2, 140.8, 135.6, 127.4, 122.4, 81.7, 67.5, 64.2, 55.4, 50.7, 39.9, 36.5, 29.3, 2.6. Anal. ($C_{22}H_{36}IN_4O_{12}PS$), C, H, N.

Similar treatment of **Ib-15E** (405 mg) with TFA (6 mL) and crystallization of the product from CH_2Cl_2/petroleum ether gave diacid **Ib-15P** as a yellow solid (306 mg, 89%): mp 147-150 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.93 (m, 1 H), 8.74 (d, J = 2.8 Hz, 1 H), 8.36 (d, J = 2.8 Hz, 1 H), 4.27 (m, 2 H), 4.00 (m, 2 H), 3.46 (m, 6 H), 3.31 (m, 2 H), 3.12 (s, 3 H). ^{13}C NMR δ 165.5, 145.6, 145.2, 140.8, 135.7, 127.6, 122.3, 67.6, 63.3, 55.5, 50.7, 39.9, 36.5, 2.7. Anal. ($C_{14}H_{20}IN_4O_9PS$), C, H, N.

Example 29. 2-({3-[Bis(2-bromoethyl)amino]-2,6-dinitrobenzoyl}amino)ethyl dihydrogen phosphate (Ic-8P). Similar phosphorylation of alcohol **IIc-8** (1.41 g, 2.83 mmol) with di-*tert*-butyldiethylphosphoramidite (93%, 1.25 g, 5.0 mmol), followed by flash column chromatography on silica gel, eluting with EtOAc/petroleum ether (1:1), gave **Ic-8E** as a yellow solid (1.77 g, 91%): mp (EtOAc/petroleum ether) 112-114 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.86 (m, 1 H), 8.24 (d, J = 9.4 Hz, 1 H), 7.63 (d, J = 9.4 Hz, 1 H), 3.92 (m, 2 H), 3.70 (m, 4 H), 3.60 (m, 4 H), 3.22 (m, 2 H), 1.78 (m, 2 H), 1.41 (s, 18 H). ^{13}C NMR δ 161.4, 145.9, 139.9, 137.3, 129.2, 127.8, 122.5, 81.3, 64.1, 52.5, 35.9, 30.1, 29.4. 29.1. Anal. ($C_{22}H_{35}Br_2N_4O_9P$), C, H, N.

Similar treatment of **Ic-8E** (900 mg) with TFA (10 mL) gave diacid **Ic-8P** as a yellow foam (754 mg, 100%): ^{1}H NMR [$(CD_3)_2SO$] δ 8.83 (m, 1 H), 8.24 (d, J = 9.4 Hz, 1 H), 7.63 (d, J = 9.4 Hz, 1 H), 3.86 (m, 2 H), 3.73 (m, 4 H), 3.60 (m, 4 H), 3.22 (m, 2 H), 1.76 (m, 2 H). ^{13}C NMR δ 161.3, 145.9, 140.1, 137.4, 129.2, 127.6, 122.5, 62.9, 52.5, 36.0, 30.0, 29.3. HRMS (FAB) calcd for $C_{14}H_{20}{}^{79}Br_2N_4O_9P$. $[M+H]^+$ *m/z* 576.9335, found 576.9326.

Example 30. 2-[(2-Bromoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate (Ic-13P). Similar phosphorylation of **IIc-13**, followed by chromatography of the product on silica gel and elution with CH_2Cl_2/EtOAc (1:3), gave 2-[(2-bromoethyl)-3-(7-*tert*-butoxy-9,9-dimethyl-7-oxido-6,8-dioxa-2-aza-7-phosphahex-1-anoyl)-2,4-dinitroanilino]ethyl methanesulfonate **(Ic-13E)** (70%) as a yellow solid: mp (CH_2Cl_2/i-Pr_2O) 95-96 °C (dec). ^{1}H NMR [$(CD_3)_2SO$] δ 8.83 (t, J = 5.6 Hz, 1 H), 8.26 (d, J = 9.4 Hz, 1 H), 7.65 (d, J = 9.4 Hz, 1 H), 4.28 (t, J = 5.3 Hz, 2 H), 3.92 (q, J = 6.7

Hz, 2 H), 3.72-3.62 (m, 4 H), 3.62-3.55 (m, 2 H), 3.23 (q, J = 6.5 Hz, 2 H), 3.15 (s, 3 H), 1.79 (pent, J = 6.7 Hz, 2 H), 1.42 (s, 18 H). Anal. ($C_{23}H_{38}BrN_4O_{12}PS$) C, H, N, P.

Similar treatment of ester **Ic-13E** with TFA gave diacid **Ic-13P** (98%) as a hygroscopic yellow solid. ^{1}H NMR [$(CD_3)_2SO$] δ 8.84 (t, J = 5.7 Hz, 1 H), 8.26 (d, J = 9.4 Hz, 1 H), 7.65 (d, J = 9.4 Hz, 1 H), 4.28 (t, J = 5.3 Hz, 2 H), 3.88 (q, J = 6.8 Hz, 2 H), 3.72-3.62 (m, 4 H), 3.53 (after D2O exchange, t, J = 6.0 Hz, 2 H), 3.23 (q, J = 6.6 Hz, 2 H), 3.15 (s, 3 H), 1.76 (pent, J = 6.7 Hz, 2 H). HRMS (FAB) calcd for $C_{15}H_{23}{}^{79}BrN_4O_{12}PS$ $[M+H]^+$ *m/z* 592.9954; found 592.9956. Treatment of diacid **Ic-13P** with $NaHCO_3$ (1:0 equiv) gave the monosodium salt.

Table 2. Combustion analysis data for new compounds of Tables 1a and 1b

No	Found				Calculated			
	C	H	N	other	C	H	N	other
IIa-1	44.5	3.9	18.6		44.6	4.1	18.9	
IIa-3	41.3	4.3	13.7	17.4 (Cl)	41.1	4.4	13.7	17.3 (Cl)
IIa-7	32.6	3.3	11.6	33.3 (Br)	32.3	3.3	11.6	33.0 (Br)
IIa-7s	33.4	3.7	7.8		32.5	3.7	8.1	
IIa-8	33.9	3.6	11.4	32.1 (Br)	33.8	3.6	11.3	32.1 (Br)
IIa-9	35.5	3.8	10.7	31.2 (Br)	35.2	3.9	10.9	31.2 (Br)
IIa-14	27.3	2.6	9.6	43.8 (I)	27.0	2.8	9.7	43.9 (I)
IIb-1	51.2	5.7	15.9		51.3	5.7	15.9	
IIb-3	41.6	4.5	13.6	17.1 (Cl)	41.1	4.4	13.7	17.3 (Cl)
IIb-7	32.9	3.3	11.5	33.3 (Br)	32.3	3.3	11.6	33.0 (Br)
IIb-7a	35.3	3.8	10.9		35.2	3.9	10.9	
IIb-8	34.9	3.7	11.3	32.3 (Br)	33.8	3.6	11.3	33.3 (Br)
IIb-14	27.8	3.1	9.5		27.0	2.8	9.7	
IIc-12	33.8	3.7	11.0		33.7	3.8	11.2	
IIc-13	35.4	3.9	11.0		35.2	4.1	10.9	
IIc-14	36.7	4.5	10.2		36.4	4.4	10.6	

Ib-7E	37.7	4.9	8.3	4.6 (P)	37.3	4.9	8.3	4.6 (P)
Ib-2E	44.8	6.2	9.0	5.1 (P)				
Ib-14E	32.9	4.2	7.2	3.8 (P)	32.7	4.3	7.3	4.0 (P)
Ia-3E	44.2	5.9	9.3		43.9	5.9	9.3	
Ia-8E	38.5	5.0	8.2		38.3	5.1	8.1	
Ic-13E	39.0	5.4	8.9	4.4 (P)	39.2	5.4	7.9	4.4 (P)

Representative alcohols of Formula I (listed in Table 1a) show selective cytotoxicity towards human cancer cell lines tranfected with either the E. coli nitroreductase cDNA (NTR) (Table 3, columns 2 and 3), or human cytochrome P450 reductase (P450R) under hypoxic conditions (Table 3, columns 4 and 5). In this table, sensitivity ratios are displayed to indicate the degree of selectivity for either NTR expression (column 3) or hypoxia (column 5). The presence of P450R is not mandatory for hypoxia selectivity. IC_{50} values are derived from 5-day proliferation cell culture experiments using the sulphorhodamine B assay and measure the concentration of prodrug required to inhibit growth to 50% of control.

Table 3. Selective cytotoxicities of representative examples of the alcohols of Table 1a

No	**Human colon (4 h)**		**Human lung (4 h)**	
	WiDr (NTR^{+ve}) IC_{50} (μM)	WiDr WT:NTR IC_{50} Ratio	**A549 ($P450R^{+ve}$)** anoxia IC_{50} (μM)	**A549 ($P450R^{+ve}$)** O_2/AnO_2 IC_{50} Ratio
IIa-1	5.2	34	3.7	28
IIa-2	48	26	25	3.7
IIa-3	47	36	54	23
IIa-7	1.5	99	6.7	49
IIa-7s	9.3	35	2.1	109
IIa-8	1.6	224	23	6.6
IIa-9	6.4	58	22	9.4

IIa-10	10	22	-	-
IIa-11	11	9	-	-
IIa-12	4.2	116	73	10
IIa-13	5	90	32	18
IIa-14	2.9	49	13	4.5
IIb-1	61	2	384	<1.3
IIb-2	11.8	47	18	20
IIb-3	13.6	59	30	9
IIb-4	14	18	-	-
IIb-5	13	19	-	-
IIb-6	27	5	-	-
IIb-7	0.3	61	0.8	56
IIb-7a	0.5	27	1.0	5.3
IIb-8	0.4	13	1.1	24
IIb-9	0.9	5	1.4	20
IIb-10	0.9	2	2.3	11
IIb-11	1.0	2	6.6	4.5
IIb-12	0.4	48	0.28	133
IIb-13	0.3	27	0.15	138
IIb-14	0.8	12	1.0	27
IIb-15	0.3	31	0.28	118
IIc-7	10	46	3.9	40
IIc-8	5.0	70	6.6	24
IIc-9	31	6	7.3	21
IIc-12	5.0	84	2.6	173
IIc-13	4.3	95	4.5	134
IIc-14	20	16	7.1	57

The activity of the phosphates hypoxic cytotoxins is demonstrated by the data in Figure 1 for the representative example (**Ib-7P**). This employs the Rif-1 *in vivo* excision assay, where the oxic tumour cells are sterilised using 15Gy of radiation, and the cytotoxicity of an agent against the remaining hypoxic cells can be quantitated. Unexpectedly, the activity of the phosphate **Ib-7P** is found to exceed that of its parent alcohol (**IIb-7**) at their respective maximum tolerated doses (750 μMol/kg).

Figure 1. *In vivo* activity of **IIb-7** and its corresponding phosphate pre-prodrug **Ib-7P**, relative to the known hypoxic cytotoxin tirapazamine.

Figure 1a.

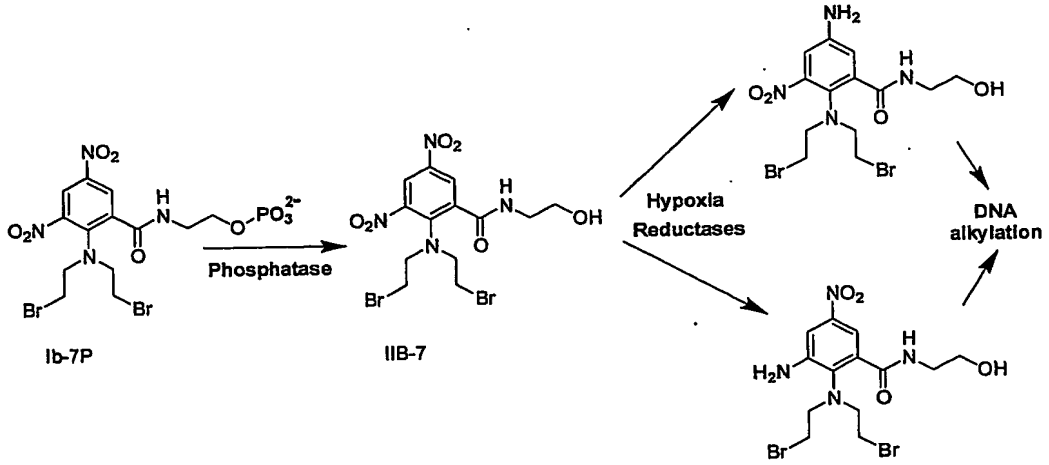
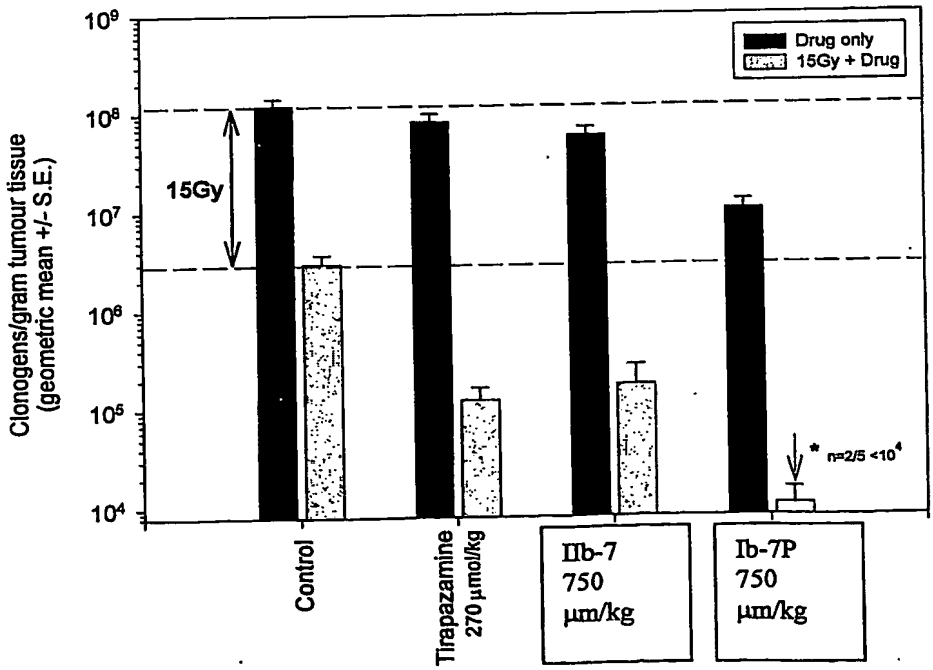


Figure 1b.

Excision assay method: 300-500mg Rif-1 xenograft → 15Gy → Single prodrug dose @ MTD → 18hr → excision → disaggregate → plate 10^2-10^5 cells → 12 days → colonies counted.



A representative example of the phosphates of Formula I as NTR activated cytotoxins is provided in Figure 2. In the WiDr *in vivo* growth delay assay, xenografts containing mixtures of $WiDr^{WT}$ and $WiDr^{NTR}$ cells are grown to $300mm^3$ and treated with a single dose of prodrug at its MTD. Tumour growth is monitored over time and animals are euthanased when mean tumour volume $>1600mm^3$. Data is presented as time to death. Unexpectedly, the activity of the phosphate (Ib-7P) is observed to exceed that of its parent alcohol (IIb-7), when administered at their respective maximum tolerated doses (750 μMol/kg). Ib-7P is superior to IIb-7 with respect to (i) time to first treatment failure (77-days vs. 17-days) and (ii) over-all survival (40% vs. 6%).

Figure 2. *In vivo* activity of IIb-7 and its corresponding phosphate pre-prodrug, Ib-7P, against human colon carcinoma xenografts containing 10% NTR+ve cells.

Figure 2a.

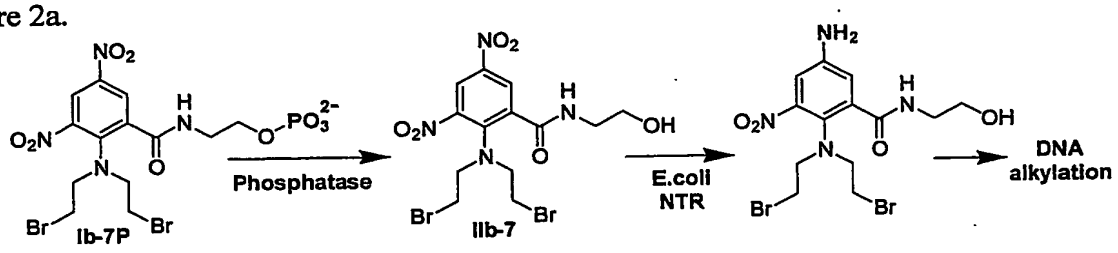
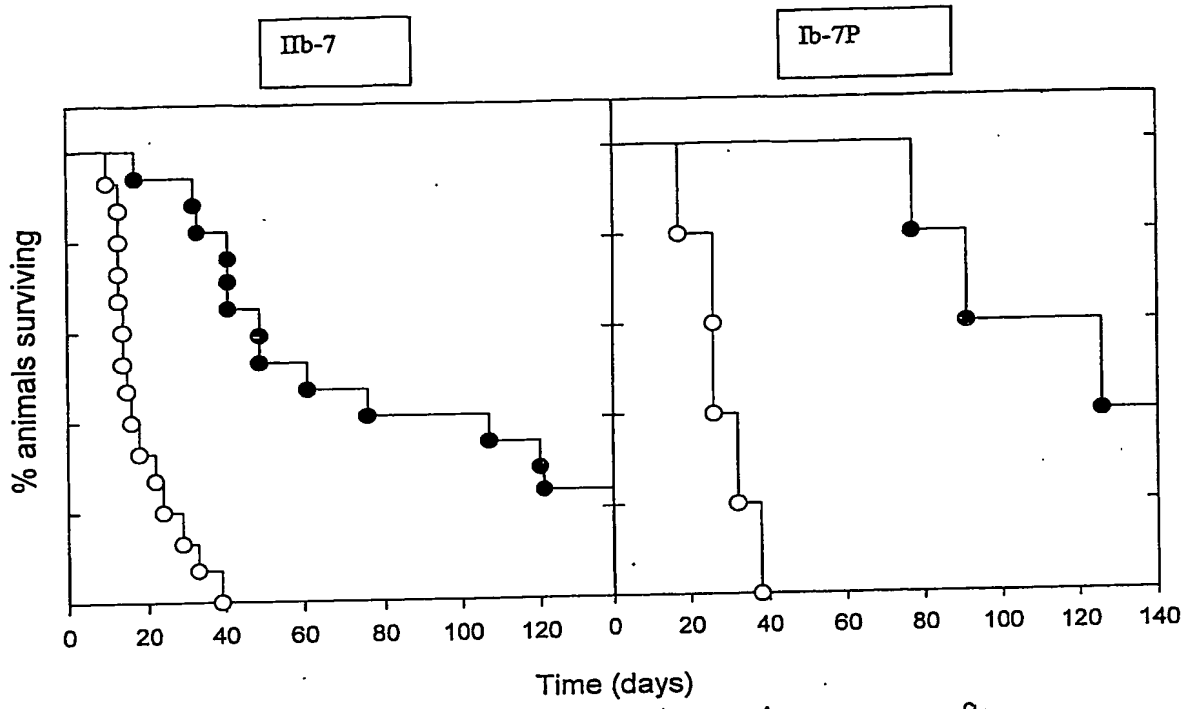


Figure 2b.



Legend: Open circles: wild-type WiDR human colon carcinoma xenografts
Filled circles: WiDr cells transfected with the *E. coli* NTR gene.

Wherein the foregoing description reference has been made to reagents, or integers having known equivalents thereof, then those equivalents are herein incorporated as if individually set forth.

While this invention has been described with reference to certain embodiments and examples, it is to be appreciated that further modifications and variations may be made to embodiments and examples without departing from the spirit or scope of the invention.

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